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THE LIFE HISTORY OF POLYSIPHONIA VIOLACEA.

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY. LXXXVII.

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(WITH THREE DIAGRAMS AND PLATES XIX–XXVIII)

INTRODUCTION.

THERE have been published by various authors many accounts of investigations on the red algae treating of the morphology of the thallus, the development of the cystocarp, and tetraspore formation. The first general studies on the reproductive processes in the group were those of BORNET and THURET (12) and JANCZEWSKI (43). These papers have never been surpassed in clearness of expression and beauty of illustration, but they considered simply the outer morphology or histology and gave no cytological details of fertilization, nor did they trace the life history. SCHMITZ (69) published an account of the fructifications of more than forty species in various groups of the red algae, giving special attention to the auxiliary cells, but in his conclusions he failed to distinguish between the act of fertilization and the secondary fusions concerned with the auxiliary cells, and he developed elaborate speculations in which these fusions were included as a part of the sexual process. This misconception was cleared up by OLTMANNS' discovery (55) that the real sexual act is the union of male and female gamete nuclei in the carpogonium, and that the auxiliary cells are probably only concerned with the nourishment of the cystocarp. OLTMANNS was the first author to develop the theory that the structure derived from the fertilized carpogonium was sporophytic in character; however, he presented no cytological evidence for this view. WOLFE (**86**) placed this theory of OLTMANNS on a cytological basis by showing that the cystocarp of Nemalion contained nuclei with double the number of chromosomes found in the sexual plants or gametophytes. However, WOLFE did not give a detailed account of the period of chromosome reduction. Nemalion is one of the simplest types of the red algae. There are no auxiliary cells or tetraspores, at least on the American plants so far as known; consequently the life history is very much simpler than that of the higher forms. The behavior of the auxiliary cell nucleus during the development of the cystocarp has been studied by OLTMANNS (**55**) with especial clearness in Callithamnion and Dudresnaya, but the structural difference between the nuclei of auxiliary cells (gametophytic) and those derived from the fertilized carpogonium (sporophytic) was not determined by him. Moreover, as regards the real nature of the tetraspore, so characteristic of the red algae, there has been no cytological work except a study of nuclear division in Corallina by DAVIS (**18**).

The significance of the tetraspore in the life history was not known. Various authors have presented speculations upon the subject; for example, OLTMANNS (**55**) regarded the tetraspore as an asexual reproductive structure comparable to brood organs or gemmae, having no fixed place in the life cycle, and STRASBURGER has followed this interpretation.

This investigation was begun in the hope that some of these problems might be solved by carefully following the life history of a type with particular attention to the behavior of the nucleus at critical periods. Although red algae include a wide range of types, the nature of the tetraspore and the history of the life cycle where tetraspores are present have probably been determined by this investigation of the ontogeny of *Polysiphonia violacea* Grev., except in forms where abnormalities may be present, due perhaps to apogamy or apospory.

As stated in a preliminary paper (YAMANOUCHI **87**), the material was collected at Woods Hole, Mass., during July and August 1905, where cultures of the carpospores and tetraspores were made to obtain stages in their germination. The method of killing, fixing, imbedding, cutting, and staining are given in that preliminary note.

This paper presents first the results of my studies of the mitosis in germinating tetraspores and carpospores and in the vegetative cells of mature plants; then comes an account of spermatogenesis, formation of procarp, fertilization, and development of the cystocarp; tetraspore formation is then considered, followed by a description of certain abnormalities; finally, there is a discussion of the cytological phenomena and alternation of generations. This last topic has been given considerable attention, for the chief results of this investigation have been the establishment of an antithetic alternation of generations in Polysiphonia, with the period of chromosome reduction at the time of tetraspore formation.

The investigation was begun during the summer of 1905 at the suggestion of Dr. BRADLEY M. DAVIS during my stay as an occupant of a Carnegie research table at the Marine Biological Laboratory, Woods Hole; and to the Carnegie Institution I wish to express my obligations for the privilege of the table. The studies were continued and completed by me in the Hull Botanical Laboratory as a Fellow of The University of Chicago, under the direction of Professor JOHN M. COULTER and Dr. CHARLES J. CHAMBERLAIN, the kind assistance and painstaking criticism of Dr. DAVIS continuing also throughout the whole progress of the investigation. To these gentlemen I am under great obligation; and also to the other members of the botanical staff of Hull Botanical Laboratory for courtesies extended to me in many ways.

THE FIRST MITOSIS IN THE GERMINATING TETRASPORE.

The tetraspore when discharged from the parent plant assumes a spherical form. Plastids usually lie near the periphery of the cell, whose cytoplasm presents an irregular, coarse alveolar structure, with the nucleus lying near the center. The cytoplasm surrounding the nuclear membrane is a finer network than anywhere else in the cell. Within the nucleus there is a very delicate linin mesh dotted here and there with chromatic granules (*fig. 1*). From the fact that the transverse walls of the cytoplasmic alveoli end on the nuclear membrane at points where the linin threads start, it seems possible that there exists a close physiological relation between these structures. The nucleus generally contains a single nucleolus, variously situated and homogeneous in structure, but sometimes two nucleoli are present.

Preliminary to mitosis, the delicate network becomes somewhat coarser and the thread somewhat broader, and gradually in many different parts the chromatin granules appear in irregular rows as chains of beads of different lengths. There are about 20 of these chains, as illustrated in *figs. 2a–2c*, which represent three sections of the same nucleus. They are the beginnings of the chromosomes, similar to the prochromosomes described by OVERTON (**58**) in the presynaptic stage in the pollen mother cells of Thalictrum and three other species of flowering plants. The material which accumulates in these prochromosomes must come from the chromatin granules imbedded in the linin thread, for it is evident that the nucleolus does not contribute any material directly to their formation. This behavior is therefore similar to the process of chromosome formation in higher plants, and is very different from the condition reported by WOLFE (**86**) for Nemalion, where the chromosomes are described as coming out from the nucleolus. The nucleolus of Polysiphonia remains unchanged while the prochromosomes are being formed. These prochromosomes gradually become more pronounced, increase in breadth, and the bead-like structure is transformed into the more homogeneous rod-shaped chromosomes that become distributed through the whole nuclear cavity attached to a linin thread, as shown in *figs. 3a* and *3b*, which represent two sections of the same nucleus. The nucleolus may remain undivided or fragment into two at this time.

The cytoplasm around the resting nuclear membrane appears at first homogeneous, but during prophase there is a gradual accumulation on the two opposite sides of the nucleus, and finally two deeply staining centrosome-like bodies appear, forming the poles of the more slightly elongated nucleus (*fig. 4*). While these changes in the cytoplasm are going on without the nucleus, some important events take place within. The chromosomes become thickened and more compact and gather in the middle region of the nuclear cavity, with linin threads still attached to their ends, and at last they are arranged in the equatorial plate (*fig. 5*). The nuclear membrane is still present when the spindle is developed (*fig. 5*), so that the latter is consequently intranuclear.

It is very interesting to compare this stage with the previous one (*fig. 4*), taking into consideration the kinoplasmic centers, the shape of the nuclear cavity, and the spindle. As regards the centers of kinoplasmic activity, in the preceding stage the kinoplasm is shown in the process of accumulation at the two poles, and a single clearly differentiated granule may be interpreted as the first visible indication of approaching spindle formation. At the time when the equatorial plate is formed, the kinoplasmic material becomes massed more densely than before, and two very large centrosphere-like structures are differentiated at the poles of the spindle (*figs. 5, 6*). These kinoplasmic bodies have a compact, well-defined form, but are without radiation. The nuclear cavity at the stage of metaphase is smaller than before, and the poles of the spindle become drawn closer together. The development of the spindle proved very difficult to study, and its history is discussed later in the paper under the head of spindle formation.

The accumulations of kinoplasm at the poles of the spindle are very characteristic and resemble the centrospheres described by DAVIS (**18**) in the tetraspore mother cell of Corallina, except that the latter have well-defined radiations. The chromosomes when arranged in the equatorial plate are readily counted if viewed in transverse sections of the spindle (*fig. 7*), as well as during prophase (*figs. 3a, 3b*), and the number is clearly 20. Granular fragments of the nucleolus are always present in the nuclear cavity during the metaphase, after which they disappear.

The duration of metaphase is rather long and the centrosphere-like structures persist until late anaphase. When each group of daughter chromosomes passes to the pole of the spindle, there are left only a few fibrils forming a central spindle between them (*fig. 8*). After anaphase the kinoplasm intrudes into the nuclear cavity and the central spindle gradually disappears (*fig. 9*). The kinoplasm thus surrounds the groups of daughter chromosomes, and the centrosphere-like structure loses its distinct differentiation and becomes a cloudy mass of kinoplasm without a clearly defined boundary (*fig. 9*).

Each group of daughter chromosomes, which during anaphase had a flattened form, becomes more or less spherical, with a small space within (*fig. 10*). The mass of chromosomes surrounded by granular kinoplasm comes to lie in nuclear sap or caryolymph, and it seems possible that the nuclear membrane may be formed as the result of the contact of the caryolymph with the surrounding cytoplasm (*fig. 11*). Such conclusions were drawn by LAWSON (44) and GRÉGOIRE and WYGAERTS (36) in their studies of the telophase of mitosis. The chromosomes later lose their individual outlines and the mass becomes transformed into a chromatin network (*fig. 12*). A new nucleolus is then formed in the daughter nucleus.

No mention has yet been made of the manner of cell division. The coarse alveolar structure of the cytoplasm taken as a whole persists during mitosis, the kinoplasm associated with the division of the nucleus remaining distinct from the alveolar cytoplasm and reacting more deeply to the plasma stains. The daughter nuclei when formed lie above one another in the germinating tetraspore. Before they have attained their full size a cleavage furrow appears at the middle region of the cell which is at first very shallow. The central spindle that lay between the two groups of daughter chromosomes has entirely disappeared before the cleavage furrow is formed, so that the center of the cell is filled by cytoplasm which presents a very coarse alveolar structure, especially in the middle region, where the cleavage furrow begins (*fig. 13*). This furrow proceeds inward, the only visible assistance in its development being the extensive fusion of vacuoles by the breaking of their limiting membranes so that less resistance is presented to its progress. Finally, the furrow reaches nearly to the center of the cell (*fig. 14*), so that the tetraspore becomes divided into daughter cells, which are in communication by a strand of protoplasm, as is so generally characteristic of the red algae.

THE FIRST MITOSIS IN THE GERMINATING CARPOSPORE.

The carpospore on its escape from the cystocarp is somewhat pear-shaped, but it gradually assumes an oblong or spherical form while floating in the water. The coarse alveolar structure of the cytoplasm, the arrangement of the plastids, and the fine linin network within the nucleus (*fig. 15*) are similar to those of the tetraspore. Moreover, the first mitosis takes place at about the same period after their escape from parent plants, namely after about fifteen hours.

The history of the mitosis in the germinating carpospore is so similar to that in the tetraspore that it seems best to point out only the marked differences between the two. The delicate linin network within the nucleus passes into a coarse chromatin reticulum upon which chains of chromatin granules appear here and there (*figs. 16a–16c*), and these are prochromosomes, as illustrated in the preceding figures of the tetraspore (*figs. 2a–2c*). The number of prochromosomes, however, is 40, and consequently double the number in the germinating tetraspore. The 40 prochromosomes grow more and more homogeneous in structure and finally become elongated chromosomes (*figs. 17a, 17b*). The weakly staining linin network disappears, but short threads remain attached at the ends of chromosomes. The kinoplasm surrounding the nuclear membrane becomes accumulated at the two poles of the nucleus, where a centrosome-like body may always be found (*fig. 18a*), and this accumulation of kinoplasm proceeds still further until there are two conspicuous centrosphere-like structures differentiated at metaphase (*fig. 19*). The spindle is somewhat larger and broader than that in the tetraspore, because of the double number of chromosomes (*fig. 19*). The polar view of that stage (*fig. 20*) clearly shows the number 40.

The nucleolus fragments during metaphase, the portions lying beside the spindle (*fig. 19*) and sometimes remaining until anaphase, after which they disappear. The behavior of the daughter chromosomes after anaphase is the same as during mitosis in the tetraspore; the groups of daughter chromosomes gather at the poles of the spindle (*fig. 21*) and become surrounded by granular kinoplasm (*fig. 22*). At the time of the formation of the nuclear membrane, the chromosomes may still be recognized and estimated as 40 (*figs. 23a, 23b*). The daughter nuclei increase in size by the secretion of nuclear sap (*figs. 24a–24c*), and finally the chromatin becomes distributed over a linin network in the resting nucleus (*fig. 25*). The germinating carpospore becomes divided by a cleavage furrow in a similar manner to that of the tetraspore.

The second and third mitosis in both germinating tetraspore and carpospore were also studied, and they were similar to those of the first divisions, showing always the two essential differences in the number of chromosomes.

MITOSIS IN THE VEGETATIVE CELLS OF THE MALE, FEMALE, AND TETRASPORIC PLANTS.

To make sure of the number of chromosomes contained within the nuclei throughout the life history, the mitoses in vegetative cells of the three forms of Polysiphonia plants—male, female, and tetrasporic—were studied. The following is a very brief account of the essential features of these mitoses.

The nuclei in the apical cells of any of the three forms of plants are somewhat larger in size than those in older region of the thallus; but although it is not difficult to obtain the successive stages of mitosis in older parts, the nucleus of the apical cell is somewhat more favorable for study and will be used in this description.

The cytoplasm in the apical cell shows very fine alveolar structure, the plastids lie near the wall, and the nucleus in the resting stage resembles that in the germinating carpospore and tetraspore (*figs. 26, 45*). The linin network becomes coarser (*figs. 27, 46*), and finally in the case of the male (*figs. 28, 29*) and female plants (*fig. 36*) 20 chromosomes appear, whereas in the tetrasporic plant (*fig. 47*) 40 chromosomes are present. The chromosomes may be readily counted at metaphase in polar views of equatorial plates, when it is evident that the sexual plants have 20 (*figs. 31, 38*) and the tetrasporic plants 40 (*fig. 50*). In spite of the small size of the nuclei, kinoplasmic accumulations at opposite poles of the nucleus are evident during prophase, and deeply staining centrosome-like bodies are conspicuous at the poles just before the spindle is formed (*figs. 29, 36*). Centrosphere-like structures are very conspicuous at the poles of the spindle during metaphase (*figs. 30, 37, 48*). These structures are more clearly shown in the mitosis in older regions of the thallus. *Fig. 43* illustrates such a mitosis from a female plant, those of the male and tetrasporic plants being omitted to avoid repetition. The smaller size of the nuclear cavity during metaphase is as constant a character of these mitoses as of those in the tetraspores and carpospores. After metaphase the two sets of daughter chromosomes remain included in the old nuclear membrane for a while (*figs. 32, 39, 49*). During the anaphase the groups become further separated, the nuclear membrane disappears, and a large vacuole intrudes between them (*figs. 33, 41, 51*). When the daughter nuclei are completely formed, a cleavage furrow develops at the periphery in the middle region of the cell (*figs. 35, 42*), a large vacuole being present in the center. The mechanism of constriction by the cleavage furrow is probably greatly assisted by the presence of this single vacuole, in place of numbers of smaller ones which are found in the tetraspores and carpospores.

SPERMATOGENESIS.

The sperms or spermatia are formed normally on special short branches called antheridia, which are developed in clusters at the tips of the main filaments. The antheridium consists of an axial siphon (*diagram 1*) which becomes surrounded and covered by a large number of small cells. These generally develop sperm mother cells at the periphery of the antheridium, and may consequently be called "stalk cells." The mitoses in the axial siphon of the antheridium were studied (*fig. 52*), as well as those which form the stalk cells (*fig. 53*), and they showed the number of chromosomes to be 20. The methods of chromosome formation, the development of the intranuclear spindle, and the cell division by constriction are the same as those of the vegetative cells already described.

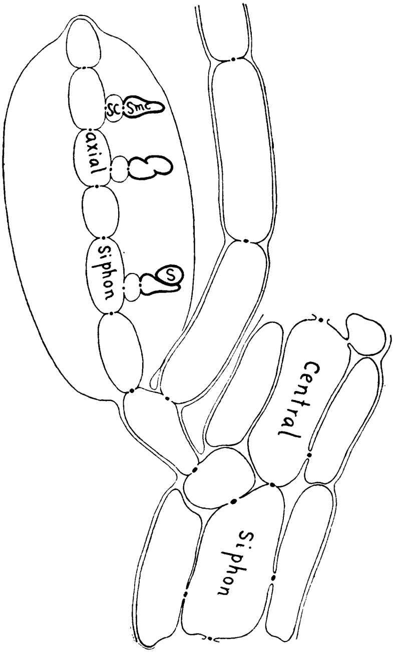


DIAGRAM 1.—Section of an antheridium, showing position of axial siphon, stalk cell (*sc*), sperm mother cell (*smc*), and development of the sperm (*s*).

The formation of the sperm mother cells from the stalk cells (*fig. 54*) is illustrated in *figs. 55–61*. *Fig. 55* presents the prophase of of the mitosis, *fig. 56* metaphase, *fig. 57* the equatorial plate viewed from a pole, *fig. 58* shows anaphase, and *figs. 59* and *60* illustrate telophase. The cell division by constriction is shown in *fig. 61*. The sperm mother cell (*fig. 62*) increases in size and assumes its characteristic form, which is narrow at the periphery and swollen at the base. In rare cases the formation of the stalk cell is omitted, so that sperm mother cells are developed directly from the axial siphon of the antheridium.

The cytoplasm of the sperm mother cell (*fig. 62*) has a very delicate granular structure and is generally destitute of plastids. The nucleus in the resting state contains a fine network and a nucleolus. Prochromosomes, 20 in number, are formed in the network (*figs. 63, 63a*) and are connected by weakly staining linin threads. The prochromosomes increase in size and become rod-shaped chromosomes (*figs. 64, 64a*).

Kinoplasm becomes differentiated from the surrounding cytoplasm and accumulates at the poles of the elongating nucleus, and a centrosome-like body appears at each pole (*figs. 64, 64a*). The stage of prophase passes into metaphase (*figs. 65, 65a*), when centrosphere-like structures are well-developed and the axis of the spindle is shorter than the diameter of the equatorial plate, as is the case during the mitoses within the carpospores, tetraspores, and vegetative cells. The number of chromosomes is clearly 20 in this mitosis, as shown in polar view of the equatorial plate (*fig. 66*). The nuclear membrane is present during metaphase (*fig. 67*), and as the two groups of daughter chromosomes separate a vacuole intrudes between them (*figs. 68, 69, 70*). The centrosphere-like structures are not recognizable after metaphase. The set of daughter chromosomes which passes to the basal region of the cell becomes aggregated, surrounded by a nuclear membrane, and enters into a resting condition; while the chromosomes of the other set, passing to the upper part of the cell still retain their individuality, although it is probable that a very delicate membrane may be formed (*fig. 70*).

A cleavage furrow in the middle region of the cells appears (*fig. 71*), and by the same mechanism as in the case of vegetative cells effects a separation of the upper half as a sperm cell from the lower half. The greater part of the large vacuole is included in the sperm, which consequently has a relatively small amount of protoplasm in comparison with its size (*figs. 72, 73*). The cleavage furrow which cuts off the sperm cell crosses the sperm mother cell obliquely, and consequently the sperm assumes a lateral position, allowing the sperm mother cell to elongate. When the matured sperm is detached completely from the sperm mother cell, the latter has assumed again its characteristic extended form (*fig. 75*).

The sperm when detached is oval in form (*fig. 73*), and has a thin wall derived from the mother cell, and a large vacuole occupies almost all the cell cavity. The cytoplasm is therefore forced to lie in a thin layer under the cell wall, and the nucleus occupies the larger end of the oval. The chromosomes maintain their individuality and are connected with one another by delicate linin threads (*figs. 74, 74a*).

The nucleus remaining in the sperm mother cell after the formation of the first sperm divides at once, passing through prophase (*fig. 75*), metaphase (*figs. 76, 78*), and anaphase stages (*figs. 79, 80*), following the same history as in the previous mitoses. Here is apparent also the same conspicuous difference in the form of the nucleus between prophase and metaphase (*figs. 75, 76*), 20 chromosomes (*fig. 77*) always appearing in this critical stage. Finally, the telophase of mitosis is followed by cell division through a cleavage furrow, which cuts off the second sperm (*fig. 81*) in a similar manner to the first. The nucleus which remains in the sperm mother cell may repeat the process, forming a third sperm.

The successive formation of sperms by constriction from the sperm mother cell may be compared, in a general way at least, to the process of formation of conidia in certain groups of fungi, where the conidia are developed successively by constriction from a conidiophore. Of course such a comparison is a superficial one, since conidia are by no means comparable to sperms in the phylogenetic sense. The spermatia found in the rusts and lichens, and certain antheridia of the Laboulbeniaceae present greater resemblances. THAXTER (**78**) describes an exogenous method of sperm formation in Ceratomyces and Zodiomyces, in which sperms are developed successively from a definite point at the distal end of fertile cells of the antheridial branches, agreeing thus with the process in Polysiphonia.

WOLFE (**86**) considers the sperm of Nemalion to be the homologue of an antheridium because the sperm nucleus divides into two. No mitosis was found in the sperm of Polysiphonia, although this matter received careful attention. The sperm of Nemalion also escapes as a naked or thin-walled protoplast from the parent cell-membrane, while that of Polysiphonia becomes detached and retains the parent cell wall. The differences, however, do not seem to the author to affect the relationship of these two sperms as homologous structures. That of Polysiphonia is also the homologue of a unicellular antheridium, in which mitosis, if ever present, has been suppressed, and the cell as a unit has become the male sexual element.

FORMATION OF THE PROCARP.

Development of the carpogonial branch.—The female organ or procarp consists in the beginning of a short branch of three or four cells. The most important of these is a cell of the axial siphon which lies next to the apical cell (*diagram 2, A*). This cell increases in size more rapidly than do the adjacent cells of the filament, so that it is very easy to recognize the primordium of the female organ, and divides successively to form five peripheral cells, which finally completely surround it (*diagram 2, B*). The first stage is illustrated in *figs. 82–84* and the second in *figs. 85–87*. The third and fourth divisions of the siphon cell have not been figured, but they occur in such a manner that the third and fourth peripheral cells are formed opposite each other and between the first and second (*diagram 2, B*). The fifth division gives rise to a peripheral cell between the first and the fourth, which later develops the carpogonial branch and has been called the pericentral cell.

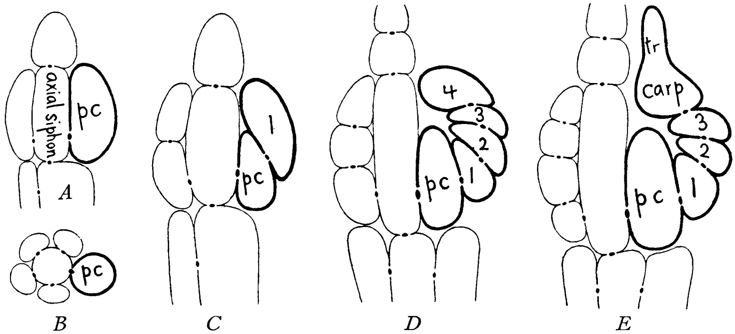


DIAGRAM 2.—Development of the carpogonial branch: *A*, young procarp with pericentral cell (*pc*); *B*, cross section of *A*; *C*, formation of first cell of carpogonial branch; *D*, the four cells of carpogonial branch; *E*, development of trichogyne (*tr*) from fourth cell or carpogonium (*carp*) of the carpogonial branch.

During every nuclear division concerned with the formation of the peripheral cells, 20 chromosomes constantly appear, as shown in polar views (*figs. 83, 86*), and this number is passed over to the pericentral cell. The nucleus in the pericentral cell divides in a direction nearly parallel to the axis of the procarp (*figs. 89–93*), cutting off a cell which develops the carpogonial branch (*diagram 2, C*). The pericentral cell later also gives rise to a group of auxiliary cells. The carpogonial branch consists of four cells which are formed successively as shown in *diagram 2, D, E.* It is somewhat bent, so that the terminal cell lies almost above the pericentral cell. This terminal cell becomes the carpogonium and develops the trichogyne. The mitoses concerned with the development of the carpogonial branch are illustrated in *figs. 94–99*, and invariably showed 20 chromosomes at metaphase. As the result of these successive mitoses, the carpogonial branch extends at one side of the central axial cell, with the pericentral cell situated between them (*diagram 2, E*).

The nucleus in the fourth or terminal cell of the carpogonial branch divides (*fig. 100*) to form two nuclei (*fig. 101*), each with 20 chromosomes, one of which becomes the female gamete nucleus, while the other enters the trichogyne that is formed at once. The upper end of the cell pushes out as a delicate process which contains almost from the beginning one of the two nuclei, the other remaining in the basal swollen region of the cell called the carpogonium (*figs. 102, 103, 104*), which corresponds to an oogonium. The formation of the trichogyne completes the development of the female organ, whose parts in longitudinal section are shown in *fig. 103*.

The trichogyne.—The trichogyne nucleus, as a rule, is situated in the middle region of the trichogyne, which has about the same breadth throughout its tubular cavity, but becomes constricted below where it joins the carpogonium. No plastids could be found in the trichogyne.

The presence of a trichogyne nucleus in the red algae has been a subject of some controversy. SCHMITZ (69) described a large single or several small granular bodies, which stained like chromatin, in the trichogyne of *Batrachospermum moniliforme* and Gloeosiphonia before fertilization, but he gives no interpretation further than the few words, "Derivate des Zellkerns der weiblichen Zellen?" Eight years later DAVIS (17) observed in the same species of Batrachospermum an unmistakable nucleus in the trichogyne, staining with haematoxylin as a dark blue body. OLTMANNS (55) also observed the granule within the trichogyne of Gloeosiphonia, but he regarded it as having no connection with the nucleus. SCHMIDLE (68) failed to find the nucleus in the trichogyne of Batrachospermum, and OSTERHOUT (57) contends that it is not present. WOLFE (86) observed a nucleus in the trichogyne of Nemalion, which he assumes to be derived from the young carpogonium, although the mitotic figure was not found. The presence of a nucleus in the trichogyne necessitates a modification of our conception of the morphology of the female organ in the red algae.

The earlier conception of the morphology of the trichogyne as given by SCHMITZ (**69**) was a cytoplasmic extension of the carpogonium, developed as the receptive organ for the sperm. This conception was followed by OLTMANNS (**55**), SCHMIDLE (**68**), and OSTERHOUT (**57**). DAVIS (**17**), however, concluded that the trichogyne is not a cytoplasmic extension from the carpogonium, but that it possesses a well-defined nucleus and hence has a certain degree of independence. The trichogyne of Batrachospermum has certainly a body that must be regarded as a chromatophore. WOLFE'S studies of Nemalion (**86**) support this view with respect to a trichogyne nucleus. In Polysiphonia, as stated above, there is present a trichogyne nucleus whose origin has been traced to a division in the terminal cell of the carpogonial branch. These facts have an important bearing on the structure of the trichogyne in lichens and Laboulbeniaceae. THAXTER (**78**) has described multicellular branching trichogynes in certain forms of Laboulbeniaceae, and the long multicellular trichogyne of Collema (BAUER **6**) and Physcia (DARBISHIRE **16***a*) illustrate similar conditions. In the lower forms of algae where heterogamy is established, male and female gametes are generally formed in unicellular antheridia and oogonia. The female gametes having become non-motile, usually remain within the oogonium and are fertilized by motile male gametes which enter the oogonium through a pore, as is illustrated by Oedogonium. With the loss of motility on the part of the male gametes, a receptive region or structure seems to have been developed by the oogonium, and in this manner the trichogyne probably arose. However, the development of the trichogyne means that the female cell, which is the homologue of an oogonium, acts as a unit. Should there be in such a cell one or more mitoses, which are the remnants of ancient nuclear division when two or more gametes may have been developed, then the supernumerary nuclei would be expected to degenerate. This seems to be the condition in the red algae, where there is an extra nucleus beside the one which is in the gamete. This extra nucleus has an important relation to the development of the trichogyne, which consequently is much more than a mere cytoplasmic extension from the carpogonium; for having a nucleus it may possess the possibilities of a somewhat extensive development. This is perhaps the explanation of the multicellular trichogynes of the lichens and the Laboulbeniaceae, which in some forms of the latter group are extensively branched.

The passage of the sperm nucleus through the trichogyne into the carpogonium.—The nucleus of the carpogonium increases in size as the female cell matures, while the nucleus of the trichogyne remains about the same size as when it was formed (*fig. 104*). The sperm becomes attached to the tip of trichogyne (*fig. 105*). The walls between the two structures dissolve, and the contents of the sperm flows into the trichogyne (*fig. 106*). The sperm nucleus consists of a number of deeply staining bodies (about 20), which are chromosomes. The nuclear membrane if present must be very delicate, for it could not be positively recognized (*figs. 106–108*). The deeply staining sperm nucleus in the trichogyne is in sharp contrast with the smaller trichogyne nucleus whose chromatin content stains weakly. The sperm nucleus moves downward, passing the trichogyne nucleus (*figs. 107, 108*), and enters the carpogonium. The female nucleus in the carpogonium, which until this time lay at the bottom of the cell, seems to move upward a short distance as if to meet the sperm nucleus (*fig. 109a*). The two gamete nuclei are strikingly dissimilar at the time of union, the male consisting of a densely packed aggregation of chromosomes, while the female is larger and in a typical resting condition, with chromatin distributed over a linin network (*fig. 109a*).

The trichogyne nucleus may still be recognized after the sperm nucleus has passed into the carpogonium. However, the cytoplasm of the trichogyne soon shows signs of disorganization, first at the tip, and a little later the trichogyne nucleus breaks down. When the male nucleus is in contact with the female and becomes somewhat pressed against it, the cytoplasm of the trichogyne has probably always separated from the carpogonium and the trichogyne has begun to shrivel.

Formation of the auxiliary cells.—Parallel with the fusion of the gamete nuclei there takes place the development of a set of auxiliary cells, as shown in *diagram 3*. The pericentral cell, which was the progenitor of the carpogonial branch and lies beneath the carpogonium (because of the growth and bending of this structure), now gives rise to two cells (*diagram 3, a1, a′1*), one somewhat below and the other at the side. The cell below divides once (*diagram 3, a′1, a′2*). The cell at the side develops a branching group which lies close beside the carpogonial branch, as shown in *diagram 3* (*a 1, a 2, a 2′, a 3, a 3′*). One of these auxiliary cells (*a 3*) is formed between the fertilized carpogonium and the pericentral cell. Thus the final result is two series of auxiliary cells, one consisting of five, the other of two cells; and in the former series it should be remembered that one of them has an important function, as will appear later, becoming the path of communication between the fertilized carpogonium and the pericentral cell.

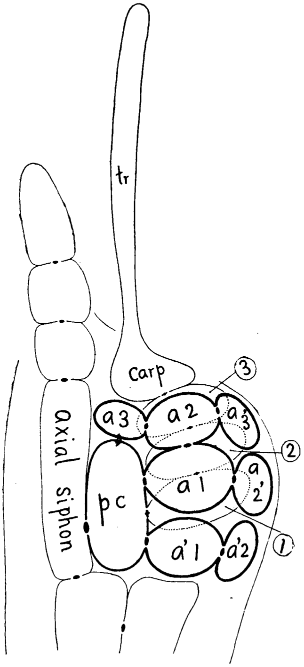


DIAGRAM 3.—Mature procarp showing arrangement of auxiliary cells: *pc*, pericentral cell; *a*1, *a*2, *a*2′, *a*3, *a*3′, *a*′1, *a*′2, auxiliary cells; 1, 2, 3, cells of carpogonial branch; *carp*, carpogonium; *tr*, trichogyne.

PHILLIPS (**60**) in his studies on the Rhodomelaceae recognized many features in the structure of the procarp of Polysiphonia which I have just described. His account of a four-celled carpogonial branch is correct, together with the general account of the formation of the central cell, as will be described presently. However, I was not able to find the arrangement of the auxiliary cells as he has described them, and his investigation lacks the cytological details through which the nuclei that enter the carpospore must be traced.

FERTILIZATION AND DEVELOPMENT OF THE CYSTOCARP.

The fusion of the gamete nuclei.—The male and female gamete nuclei which met in the carpogonium have generally fused by the time the auxiliary cells are formed. The membrane of the female nucleus appears to dissolve at its point of contact with the sperm nucleus, which lies closely pressed against it (*fig. 114a*), and later the chromatin content of the male nucleus enters the female (*fig. 115a*). The subsequent changes in the female nucleus result in the gradual transformation of its linin network into clearly defined chromatin granules and finally into chromosomes. At the same time the crowded group of male chromosomes (*fig. 115a*) becomes looser, the chromosomes separating from one another, some remaining near the periphery of the fusion nucleus, and some passing into the interior. Finally the chromosomes derived from male and female nuclei become mingled together and the fusion nucleus assumes the appearance of prophase (*fig. 116*).

The first mitosis of the fusion nucleus (*sporophytic*).—The fusion nucleus which results from the union of the male and female gamete nuclei now passes into the prophase of mitosis. The number of chromosomes is 40, which is of course double the number in the sexual plants or gametophytes, so that the fusion nucleus is sporophytic in character. These chromosomes differ from one another in size, and some of the smaller certainly come from the male nucleus. It would be interesting to trace carefully the history and behavior of these chromosomes, but I am not prepared at present to discuss this matter in detail.

The stages of prophase in Polysiphonia, as previously described, are always characterized by the presence of centrosphere-like structures at the poles; however, these structures do not seem to be present during the first mitosis of the fusion nucleus. The spindle of this mitosis is remarkable for its size and the breadth of the poles (*fig. 117a*). Another peculiarity is the fact that the nuclear membrane disappears during prophase, so that the spindle lies freely in the cytoplasm. It is possible that the early dissolution of the membrane is connected with the fusion of the gamete nuclei, which may weaken the membrane of the female nucleus. As regards the count of chromosomes, their number 40 is apparent when the equatorial plate is viewed from the pole (*fig. 118a*). In the late metaphase the same number may be estimated in both groups of daughter chromosomes (*fig. 123*), which means that this first division of the fusion nucleus is a typical mitosis. At anaphase there is present a rather conspicuous central spindle between the two sets of daughter chromosomes (*fig. 124a*).

The migration of sporophytic nuclei into the pericentral cell.—There is only one mitosis within the fertilized carpogonium. The carpogonium now fuses with the auxiliary cell, which lies between it and the pericentral cell (*diagram 3, a3*). The wall between these cells dissolves and a broad communication is formed connecting them. A fusion between this auxiliary cell and the pericentral cell follows at once, so that the carpogonium is then in free communication with the pericentral cell by means of the auxiliary cell. Sometimes this communication becomes established as early as the metaphase of the mitosis of the fusion nucleus. By means of the passage which is established by these cell unions, the two sporophytic daughter nuclei, resulting from the division of the fusion nucleus, move down into the pericentral cell (*figs. 125, 126*).

During the formation of the auxiliary cells from the pericentral cell each nucleus in the first three cells of the carpogonial branch divides (*figs. 119–122*). The daughter nuclei then lie side by side in pairs within the cells of the carpogonial branch. While the sporophytic fusion nucleus is undergoing mitosis, the protoplasmic connections between the cells of the carpogonial branch widen, and there is a movement of the cytoplasm along the branch into the carpogonium, possibly to furnish nourishment to this cell.

The communication between the carpogonium and the adjacent auxiliary cell is transient, simply furnishing a passage for the sporophytic nuclei into the pericentral cell. After their migration, the carpogonium becomes detached from the auxiliary cell and remains isolated for a while, without a nucleus, but finally breaks down with its three sister cells of the carpogonial branch.

The formation of the central cell and the development of the carpospores.—When the carpogonium becomes separated after its union with the auxiliary cell and the passage of its two sporophytic nuclei into the pericentral cell, all of the auxiliary cells become more closely united with one another. This condition takes place by the broadening of the protoplasmic communications that already exist between them. New communications are also established between neighboring auxiliary cells, so that the entire system becomes closely bound together by broad protoplasmic strands. These protoplasmic communications make possible the movement of the nuclei in the auxiliary cells towards the pericentral cell. This general cell union finally results in the formation of a large irregular cell, the central cell, as it was called by previous investigators (PHILLIPS **60**, and others), containing a number of nuclei. Two of these nuclei, as previously stated, are sporophytic and derived from the mitosis of the fusion nucleus in the fertilized carpogonium; the other nuclei, perhaps three or four in number, are gametophytic and derived from the auxiliary cells. The two sporophytic nuclei lie in the upper part of the central cell and the gametophytic nuclei below. There are not as many gametophytic nuclei in the large fusion cell as might be expected, because some of them have already broken down and the others generally show signs of disorganization.

The two nuclei of sporophytic origin give rise to a series of mitoses (*fig. 127*), and the central cell now develops several lobes, into each of which a single sporophytic nucleus generally passes (*fig. 128*). The nucleus contained within each lobe divides once more (*figs. 129, 130*) and a carpospore is cut off terminally (*fig. 131*) from the lobe by a cleavage furrow, the lower portion remaining as a stalk cell by which the carpospore is attached to the central cell (*fig. 132*).

The chromosomes appearing in the mitoses previous to the formation of the carpospore are clearly 40 in number. There is therefore no chromosome reduction at this period in the life history of Polysiphonia, for the sporophytic number 40 enters the carpospore and, as previously described, appears with the first mitosis at its germination. The period of chromosome reduction in Polysiphonia is at the time of tetraspore formation, as will be discussed presently. This is an important matter in relation to WOLFE'S (**86**) account of Nemalion, where he reports chromosome reduction as taking place just before the formation of the carpospores.

After the formation of the carpospores the central cell increases greatly in size, absorbing the stalk cells (*fig. 133*); and finally the cell of the axial siphon becomes involved in these extensive cell unions, which are probably concerned with the nourishment of the carpospores, since sixty or more, as a rule, are developed in a single cystocarp. While the carpospores are being formed, the characteristic envelop of the cystocarp becomes swollen and urn-shaped. This envelop is developed from the peripheral siphons of the procarpic branch; but is lined with a set of delicate filaments (*fig. 132, p f*), called paranematal filaments, that arise from the cell of the axial siphon.

Some of the gametophytic nuclei derived from the auxiliary cells break down before the unions or after the formation of the central cell. They swell greatly, the chromatin network becomes inconspicuous, the membrane grows thinner and finally dissolves, so that the nuclear contents mingle with the cytoplasm. Or, before the dissolution of the nuclear membrane, the network fades away, but large nucleolus-like globules appear, which after the breaking down of the membrane become distributed in the cytoplasm (*fig. 134*).

TETRASPORE FORMATION.

It is probable that true tetraspores are never formed on the sexual plants of Polysiphonia. Certain abnormalities will be discussed in the next section of this paper. The cell lineage of the tetraspore in the Rhodomelaceae was correctly described by FALKENBERG (**27**). HEYDRICH (**41, 42**) gives an account in which he contends that the tetraspores are formed after a nuclear union within the mother cell, and that tetraspore formation may be the forerunner of a method of sexual reproduction. His studies seem to have been made upon unsatisfactory material and without cytological methods, to judge from his figures. Since I have not been able to confirm his conclusions or to establish any relation between them and my own, I shall not discuss them further.

The beginning of tetraspore formation is the development of a pericentral cell laterally from the central siphon (*fig. 140, pc*). The mitosis previous to the formation of the pericentral cell (*figs. 136–139*) shows that its nucleus contains 40 chromosomes. The pericentral cell then cuts off a cell above (*fig. 140, tmc*), which becomes the tetraspore mother cell, attached by a stalk (*fig. 140, sc*) to the central siphon.

The formation of the tetraspore mother cell was traced in detail through the prophase, metaphase, and anaphase of the nuclear division in the pericentral cell (*figs. 141–146*), and the number of chromosomes which enter the tetraspore mother cell is clearly 40.

Centrosphere-like structures also appear unmistakably at the metaphase of this mitosis (*fig. 142*), as well as in the previous mitosis (*fig. 137*).

The tetraspore mother cell (*fig. 147*) increases rapidly in size, soon becoming very much larger than the stalk cell. With the growth of the cell the nucleus also increases in size, and in the resting condition shows a conspicuous linin network. This network consists at first of lightly staining anastomosing threads, having knots here and there which stain a little darker (*figs. 147, 147a*). The cytoplasm presents a fine granular structure, with small alveoli. The nuclear network undergoes gradual change in such a manner that the lightly staining threads become somewhat thicker, and the knots grow into large irregular masses (*figs. 148, 148a*). Some portions of the threads connecting the knots become thinner and more slender, at last fading away; while other portions of them become thicker, and then the knots gradually diminish in bulk; so that, by and by, the anastomosing chromatin network becomes transformed into long continuous threads of irregular thickness, finally broadening into ribbons (*figs. 149, 149a*).

These chromatin threads or ribbons derived from the network now spread and become distributed throughout the nuclear cavity in a continuous and tangled fashion, presenting no free ends. It is difficult to decide whether there is a single continuous thread or a double structure, but probably the latter condition is present, for the threads generally run side by side in pairs (*figs. 150, 150a*). Most of the chromatin threads become more tangled, twisted, and massed at one side of the nuclear cavity, only a few traversing the cavity to the opposite side of the nuclear membrane. Synapsis is now generally believed not to be an artifact, and a careful study of this stage in Polysiphonia convinces me that the uniform chromatin threads which run parallel in pairs actually fuse into a single thread in certain portions, although at the same time they may be separated in other parts. All of this probably means that the two continuous threads resulting from the transformation of the chromatin network are of distinct origin, paternal and maternal, and that they come in contact where they run closely parallel, and finally fuse together in the tangled and contracted condition of synapsis, according to the recent interpretation of ALLEN (**1**, **2**, **3**, **4**), GRÉGOIRE (**35**), BERGHS (**7**, **8**, **9**, **10**), ROSENBERG (**64**, **65**, **66**), and others.

The period of synapsis lasts for some time, after which the spirem becomes looser and distributed throughout the nuclear cavity. This thread, at first uniform in thickness, begins to split longitudinally (*figs. 152, 152a*), the two parts lying close together side by side for long distances, but sometimes diverging at wide angles and then coming together again. The transverse segmentation of this double spirem to form the chromosomes now takes place, although not simultaneously throughout the entire nucleus. The shape of these chromosome segments is very irregular when first formed. They may be bent or twisted like two Vs (*fig. 153a, V*), or two Ls (*fig. 153a, L*) placed one above the other, or crossing in the form of an X (*fig. 153a, X*). The segments gradually shorten (*fig. 154*) until 20 short rod-shaped chromosomes, bivalent in nature, are formed (*fig. 155*). These are of course really the 40 sporophytic chromosomes now grouped in pairs. The nucleolus present in the resting nucleus of the tetraspore mother cell has not changed visibly up to this prophase stage, taking various positions, and during synapsis being surrounded by the tangled mass of threads. Thus the 40 chromosomes which entered the tetraspore mother cell now appear after synapsis, which is generally believed to be the period of chromosome reduction, as 20 pairs. These pairs become arranged in an equatorial plate and the chromosomes of the pairs split longitudinally, so that a large number of chromosomes results (*fig. 156*), probably 80 in all, but so crowded that it is not possible to count them with absolute certainty.

While these changes are taking place inside of the nucleus the kinoplasm accumulates in two opposite poles outside of the nuclear membrane. Each pole of the spindle which is formed (*fig. 156*) is occupied by a deeply staining centrosome-like body, as in the case of the prophase of the other mitoses previously described. The form of this spindle in the tetraspore mother cell is quite different from other mitoses. Its longest axis runs from pole to pole instead of across the equatorial plate, as in the other nuclear figures. Besides, the two poles are less than 180° apart, which gives an asymmetrical or somewhat bent form to the spindle when viewed from a certain direction, and also there are no clearly defined centrosphere-like structures.

The spindle of the first mitosis is of very short duration. As soon as the chromosomes at the equatorial plate separate into two groups, the two spindles of the second mitosis suddenly appear and the first spindle can no longer be recognized (*fig. 157*). It is possible that the two poles of the first spindle may move along the membrane and become poles of the second spindle, but I have no evidence to support this suggestion. Whatever the origin of the poles of the second mitosis, they are placed in an entirely different position from the poles of the first. The second mitosis in the tetraspore mother cell, therefore, follows so shortly after the first that there is no period between for the organization of two resting nuclei.

The rapidity with which the second mitosis follows the first may prevent the organization of centrosphere-like structures at the poles. The axis of the two spindles of the second mitosis lie perpendicular to each other (*figs. 157, 158*), and the two nuclear divisions take place simultaneously. At anaphase four groups of chromosomes, 20 in number, pass to the poles, still included in the membrane of original tetraspore mother cell, which persists from the beginning (*fig. 159*). These four groups, of 20 chromosomes each, contain all together the 80 granddaughter chromosomes shown in *fig. 156*. When the granddaughter chromosomes reach the four poles of the two spindles of the second mitosis, four masses of kinoplasm are present. The granddaughter chromosomes after reaching the poles soon begin to lose their individual outline, and become connected with one another to form a network. (*fig. 160*). The original nuclear cavity contains at this stage a very large nucleolus-like body which appears during the anaphase of the second mitosis (*fig. 159*). The history of this body is not clear, but it seems to be a new structure, developing during the second mitosis, The four poles now begin to enlarge, while the region of the nuclear membrane between them becomes flattened (*fig. 160*); consequently the outline of the original nuclear membrane is somewhat tetrahedral at this stage. The transformation of the four groups of chromosomes into four chromatin nets proceeds until a chromatin reticulum occupies each of the four lobes of the original nuclear cavity.

The cytoplasm of the tetraspore mother cell has large alveoli, which pass into a finer structure at the periphery of the cell and at the nuclear membrane. This fine alveolar cytoplasm around the nucleus joins the kinoplasm, which lies directly against the nuclear membrane, at the four regions formerly occupied by the poles of the spindles of the second mitosis (*fig. 160*). These kinoplasmic masses extend along the membrane between the four lobes. Finally the membrane breaks down between the lobes and the kinoplasm enters the nuclear cavity in the form of fibrils (*fig. 161*), which grow slowly towards the center, where they finally meet around the nucleolus-like body which now shows signs of fragmentation. The four groups of daughter chromosomes, passing into a chromatin network, thus become surrounded by kinoplasm (*fig. 162*) and separated from one another as four daughter nuclei (*figs. 163, 163a*). During this process the nuceolus-like body, formerly occupying the center of the nuclear cavity, fragments into four or more portions, which become distributed to the four daughter nuclei.

The daughter nuclei and the tetraspore mother cell continue their growth after nuclear division, these four nuclei remaining closely associated with one another for a long while (*fig. 165*). Cleavage furrows have begun to form at the periphery of the cell a little before or after the end of nuclear division. The arrangement of these furrows may be compared to the six edges of four spherical tetrahedrons whose apices are pointed towards the center of the tetraspore mother cell. The cleavage furrows slowly grow inward (*figs. 165, 165a*), and finally meet at the center of the mother cell between the four daughter nuclei, thus dividing the protoplast into tetraspores. The mechanism of the cell division by cleavage furrows is similar to that of the vegetative cells, *i. e.*, the furrow is assisted in its growth inward by the fusion of the small vacuoles.

Throughout the whole process of tetraspore formation the mother cell remains connected with the stalk cell by a strand of protoplasm, and probably obtains nourishment through this strand, since the developing tetraspores increase greatly in size.

ABNORMALITIES.

Normally the male and female organs and tetraspores are found on three different individuals, but it often happens that antheridia and procarps are produced by the same plant. Sperms are occasionally developed in clusters at the nodal regions of older portions of filaments, more often on male plants, but sometimes they are formed near the base of cystocarps. The sexual cells in these cases develop normally, the successive mitoses showing 20 chromosomes.

Frequently certain of the stalk cells in an antheridium increase greatly in size (*fig. 166*) and divide again and again (*fig. 167*). The cytoplasmic structure and behavior of the cells resemble somewhat the auxiliary cells of the procarp, and it seems possible that there is present in such cases an organ of somewhat mixed character.

The most noteworthy abnormalities, however, are those where cystocarpic or antheridial plants produce cells whose lineage is identical with that of the tetraspore mother cell (*figs. 168–170g*). The development of these cells was traced until they reached their full size (*figs. 170a–170g*), yet the nuclei in almost all cases remained undivided (*fig. 170d′*), although the beginnings of cleavage furrows were observed as shallow grooves (*figs. 169–170g*). These cleavage furrows never proceeded to the interior of the cell. Very rarely the nucleus appeared to enter a mitosis (*figs. 171, 171a, 171a′*) in old cells, but the number of chromosomes, small and round, were about 20 in each daughter group, and there was no evidence of reduction phenomena; indeed, the cell was never divided. Whether this cell may escape from the parent plant and germinate as a monospore has not yet been determined.

It seems probable that this peculiar behavior in Polysiphonia may offer an explanation of similar cases reported in the red algae where tetraspores are formed on sexual plants. They have been noted in *Chylocladia kaliformis* (LOTSY **45**), *Spermothamnion Turneri* and *Ceramium rubrum* (DAVIS **24**), and DAVIS has also observed them on *Callithamnion Baileyi.* Such cases should be carefully investigated to determine whether true tetraspores are present or whether the structures are not really of the nature of monospores, as in Polysiphonia, and developed with a suppression of reduction phenomena. In this case the apparent irregularity of the presence of asexual spores on a sexual plant would be explained; or it is of course possible that in some cases the tetraspores are formed normally, but the sexual organs are developed apogamously. However, it seems more probable that the first alternative will be found to be the explanation of these exceptional conditions in the red algae.

DISCUSSION OF CYTOLOGICAL PHENOMENA.

The nucleolus.—The morphology of the nucleolus and its behavior and function during mitoses have been studied in some detail for two decades by many investigators in both plant and animal cells. Various conclusions have been reached by different authors for the various forms examined, and the same author has not unfrequently changed his view when he came to study different material. From the excellent comparative studies of MONTGOMERY (**48**) and work done later, it is possible to summarize the essentials of the most important views as follows.

ZACHARIAS (**88**) proposed the theory that the nucleolus contains no chromatin, after FLEMMING'S conclusion (**33**) that there were chemical differences between true nucleoli and the chromatin reticulum. This view has been followed by various authors, who have concluded that there is no relation between the formation of chromosomes and the disappearance of nucleoli. This seems to be the condition in Polysiphonia.

STRASBURGER (**72**, **74**) published the view that the substance of the nucleolus is utilized for spindle formation, which conclusion was drawn from the fact that the nucleolus in many forms disappears partly or completely, immediately preceding the formation of the spindle. Later NĚMEC (**54**) suggested that the disappearance of the nucleolus and the formation of the spindle may be regarded as two independent events, which take place simultaneously. STRASBURGER'S view has been followed by FAIRCHILD (**26**), HARPER (**38**), WILLIAMS (**81**), and others. The spindle formation of Polysiphonia will be considered in the next section under the heading "spindle formation."

STRASBURGER (**70**), however, formerly held the view that the nucleolus was reserve material serving to build up the chromosomes. This theory has been followed by PFITZNER (**59**), GUIGNARD (**37**), FARMER (**29**, **30**), SARGANT (**67**), SWINGLE (**76**), CARNOY and LEBRUN (**13**), CHAMBERLAIN (**15**, **16**), DUGGAR (**25**), ANDREWS (**5**), MOTTIER (**52**), CAVARA (**14**), WAGER (**80**), and others.

Some authors have gone still further and concluded that the chromosomes in certain forms are formed directly from the nucleolus. The studies of the algae and of lower unicellular organisms seem to support this conclusion. Thus TANGL (**77**), MEUNIER (**46**), MOLL (**49**), DECAGNY (**24***a*), HENNEGUY (**40**), MITZKEWITSCH (**47**), and VAN WISSELINGH (**83**, **84**) on Spirogyra, GOLENKIN (**34**) on Sphaeroplea, and WOLFE (**86**) on Nemalion hold this view. There are naturally some differences in details among the authors mentioned above. For example, MOLL states that the nucleolus of Spirogyra is found commonly exhibiting a skein structure, and that segments are formed by the transference of chromatin substance from the nucleolus into a nuclear plasm as small fragments arranged like beads in a necklace. MITZKEWITSCH points out that during mitosis the nucleolus increases in size and becomes differentiated into a number of deeply staining granular chromosomes. VAN WISSELINGH believes that in the same genus only two out of the eight chromosomes are derived from the nucleolus, and that in the reorganization of daughter nuclei both halves of these two chromosomes give rise to the new nucleoli. GOLENKIN describes the nucleolus of Sphaeroplea as breaking up into a number of chromosomes which become arranged in a nuclear plate. WOLFE on Nemalion states that the material of the nucleolus passes outward through radiating fibrillae (linin ?) into a number of chromatin granules, which organize the chromosomes directly without the intervention of a spirem stage.

The most recent study on Spirogyra is by BERGHS (**11**). He concludes that the nuclear network is not of chromatin nature, at least it contains in the resting state little chromatin and does not take part in the formation of chromosomes, whereas the nucleolus, at least at prophase, contains all of the chromatin elements and does not disappear at any moment of mitosis. The nucleolus consists of two substances; from the first, 12 chromosomes become differentiated and arranged in a ring at the equatorial zone; the second substance remains in the form of the original nucleolus. The second substance at anaphase splits into two groups of small rods ("bâtonnets"), forming segments which pass to the poles with the chromosomes. These segments are 6 in number, but are double longitudinally. The true chromosomes become attached in pairs at the equatorial ends of these segments.

The daughter nucleoli are reconstructed at the expense of the double segments. These undergo an active vacuolization and are then condensed into a nucleolus in which the two substance are again mixed together.

According to other views, there may be more than one kind of structure called a nucleolus. CARNOY (**12***a*) makes four groups of these structures as follows: *nucléolus nucléinieus*, *nucléolus noyaux*, *nucléolus plasmatiques*, and *nucléolus mixtes*, the first one being considered as portion of the chromatin network and the third concerned with the formation of the spindle. Other authors (ROSEN **62**, DAVIS **22**, WILSON **82**) have recognized two kinds of nucleoli, true nucleoli and chromatin nucleoli, the latter being considered entirely of chromatin.

In Polysiphonia the nucleolus lies in various positions within the nuclear cavity, and is not connected with the chromatin network. The chromosomes are formed from the gradual transformation or rearrangement of the substance of the network, and the nucleolus takes no part in their development. In Corallina also DAVIS (**18**) clearly distinguishes the chromatin bodies from the nucleolus.

Spindle formation.—The spindle fibers in Polysiphonia are meagerly developed and of short duration. During the prophase of mitosis the nucleolus remains unchanged and the two poles are marked by deeply staining bodies, but I regret that I have not been able to trace the process of spindle formation. In the sporelings, where the nucleus is comparatively large in size and the spindles are more conspicuous, it was noted that short slender fibrils are attached to the chromosomes when assembled irregularly in the middle region of the nuclear cavity (*figs. 3, 17*). These fibrils are the remains of the nuclear network and are the only fibrillar structures ever seen within the nuclear membrane at this time. The centrosome-like bodies, which at metaphase seem to become the centers of the well-differentiated centrosphere-like structures, have no radiation into the cytoplasm, though in the case of sperm mother cells a few fibrillae may be seen running from the centrosome-like bodies at prophase (*fig. 64*). I have not observed the entrance of spindle fibers from the kinoplasmic centers outside of the nucleus, as has been reported by DAVIS (**18**) for Corallina.

HARPER (39) states that, after the synaptic stage in the ascus of Phyllactinia, a number of strands of chromatin are attached to a central body, and that each strand corresponds to a single chromosome. He shows that the resting nucleus of Phyllactinia has definite polarity. The formation of chromosomes from a strand of the spirem consists in the segregation of two substances present in the spirem. The densely staining chromatin aggregates into chromosomes, leaving the achromatic portion as a series of threads connecting the chromosomes to the central body, and these threads later form the spindle. I have studied Polysiphonia very carefully in the hope of relating its process of spindle formation to that of Phyllactinia, but have not been able to find any essential resemblance. There seems to be no polarity to the resting nucleus of Polysiphonia as described by HARPER for Phyllactinia.

Centrosome and centrosphere-like structures.—Every mitosis, no matter where it occurs, is characterized by the constant presence during prophase of two sharply differentiated centrosome-like bodies in the center of the kinoplasm at opposite ends of the nucleus. When the chromosomes are arranged in the equatorial plate the kinoplasm has the form of a large centrosphere-like structure at the pole of the spindle, and the centrosome-like bodies have disappeared. These structures are destitute of the astral rays, characteristically accompanying typical centrosomes or centrospheres, as reported in Fucus (STRASBURGER 73, FARMER and WILLIAMS 32), Stypocaulon (SWINGLE 76), Dictyota (MOTTIER 52, WILLIAMS 81), and in animal cells. They have a compact, homogeneous structure in Polysiphonia, which makes them readily distinguishable from the surrounding protoplasm. The daughter chromosomes, after their separation at the equatorial plate, become gathered close to each centrosphere-like structure at anaphase of mitosis, and in contact with it. The latter then passes into a vague kinoplasmic mass which surrounds the group of daughter chromosomes.

The observations summarized above, namely the appearance of a centrosome-like body at prophase, its progressive development and differentiation as a large centrosphere-like structure during metaphase, which is the climax of the kinoplasmic activity of mitosis, and its gradual decline after anaphase, lead me to conclude that these structures in Polysiphonia are not permanent organs of the cell, but are formed *de novo* with each mitosis, to carry on the mechanism of nuclear division.

HARPER (**39**) has published an important discussion on a "central body" discovered by him in Phyllactinia. In this form the central body lies within the membrane of the resting nucleus, and is connected with chromatic strands so as to give polarity to the nucleus. The poles of the spindles are formed by division of the central body. HARPER believes in the permanence of this structure, from mitosis to mitosis, and in the maintenance of its connection with the chromatin. The permanent nature of the central body in Phyllactinia and the transient appearance of centrosphere-like structures in Polysiphonia seem at present difficult of reconciliation.

In Nemalion WOLFE (**86**) reports that centrosomes are present without astral rays at metaphase, but their continuity was not established. The centrosphere described by DAVIS (**18**) in the tetraspore mother cell of Corallina is formed *de novo* in each mitosis. Their transient nature agrees with the somewhat similar structures of Polysiphonia.

The reduction of chromosomes.—STRASBURGER'S paper (**71**) entitled "The periodic reduction of the number of chromosomes in the life history of the living organisms" was the first presentation of the significance of sporogenesis and reduction phenomena in relation to alternation of generations in plants. His conclusions were based upon the discoveries that nuclei in the sporophyte generations of higher plants have double the number of chromosomes found in the nuclei of the gametophyte generations, and that the reduction of this double number takes place at the period of sporogenesis. This theory has been well established so far as groups of plants above the thallophytes are concerned, and the period of chromosome reduction has been found to be always associated with sporogenesis, and never with gametogenesis as in the case of animals. However, among the thallophytes our actual knowledge of facts concerning the reduction period is meager.

Suggestions of the presence of reduction phenomena at gametogenesis have been made among the fungi in the Peronosporales (ROSENBERG **63**) and Saprolegniales (TROW **79**). ROSENBERG describes what he has called a synaptic stage in the nucleus preceding the two mitoses concerned with oogensis in Plasmopara, and TROW holds that chromosome reduction takes place in the two mitoses in the oogonium of Achyla. These interpretations have been discussed and criticized by DAVIS (**21**, **23**), and the suggestions of ROSENBERG and TROW do not seem to me convincing.

Among algae, one of the best known accounts of gametogenesis is that of Fucus (STRASBURGER **73**, FARMER and WILLIAMS **32**), where, although spermatogenesis has not been investigated, the history of oogenesis indicates a period of chromosome reduction. Consequently the fusion nucleus in the fertilized egg has the same number of chromosomes as the nucleus in the vegetative plants of Fucus, which led STRASBURGER (**73**, **75**) to conclude that the Fucus plant is a sporophyte generation and that the gametophyte is so greatly reduced that it is only represented by single cells —male and female gametes —before fertilization.

Other examples among the thallophytes in which the life history has been worked out in some detail are Dictyota and Nemalion. In Dictyota (WILLIAMS **81**) the fertilized egg nucleus gives rise to an asexual plant with double the number of chromosomes, and consequently a sporophytic generation. This asexual plant develops spores in groups of four accompanied by chromosome reduction, and these spores develop the gametophyte generation. This type of life history is clearly analogous to that of Polysiphonia. In Nemalion (WOLFE **86**) the fusion nucleus of the fertilized carpogonium has a double number of chromosomes which appear in all of the cells of the cystocarp (sporophytic) up to the formation of carpospores, where the reduced number of the gametophyte is reported to appear. WOLFE'S account, however, does not give the details of this chromosome reduction with the characteristic stage of synapsis followed by two successive mitoses.

Chromosome reduction in Polysiphonia is clearly similar to the phenomena of sporogenesis in higher plants, and takes place at the time of tetraspore formation. The carpospores, containing the sporophytic number of chromosomes, continue the sporophyte generation by developing the tetrasporic plant. The appearance of synapsis just previous to the formation of the tetraspores, followed by two successive mitoses with their peculiar distribution of the chromosomes, is similar in all essentials to the reduction division in the higher plants and in Dictyota.

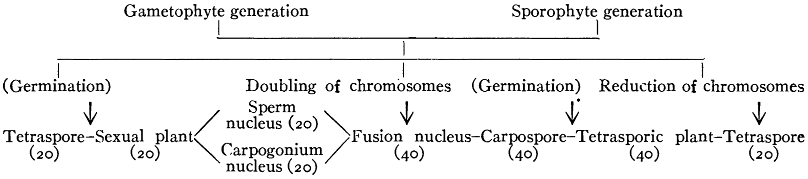
The mitoses in the tetraspore mother cell have certain peculiarities that deserve special consideration. The first mitosis is followed so rapidly by the second that there are no resting nuclei organized between the two divisions. In this respect the history of sporogenesis resembles that of Pallavicinia reported by MOORE (**50**, **51**), but there is this difference, that in Polysiphonia the granddaughter chromosomes present in the second mitosis are formed before the first and within the original membrane, and the organization of the four granddaughter nuclei takes place simultaneously. However, the distribution of the granddaughter chromosomes is clearly effected through two mitoses and two sets of spindles, so there is never present a quadripolar spindle such as was described by FARMER (**28**, **29**, **30**, **31**) for Pallavicinia and some other forms of Hepaticae, and has been called in question by DAVIS (**19**) and MOORE (**50**, **51**).

ALTERNATION OF GENERATIONS.

Alternation of generations.—Judging from the studies of Polysiphonia presented above, the male and female plants with their 20 chromosomes are gametophytes. The union of the male and female nuclei results in the fertilized carpogonium with the double number of chromosomes (40), marking the beginning of a new phase, the sporophyte generation. This fusion nucleus gives rise to a series of mitoses in the central cell of the cystocarp, all characterized by the double number of chromosomes, and consequently sporophytic in character, and carpospores are finally formed. The carpospore on germination presents the same number of chromosomes (40), and the successive mitoses following contain this number, so that the sporeling developed from the carpospore is still a part of the sporophytic phase. It may never be possible to grow such sporelings to maturity under experimental conditions, but it is evident that the plant developed from the carpospore must have nuclei with 40 chromosomes, until there is some marked change in the life history. The only vegetative form of Polysiphonia with 40 chromosomes is the tetrasporic plant, from which it must be inferred that the tetrasporic plant arises from the carpospore and constitutes a part of the sporophyte generation that begins with the fertilized carpogonium .

This long sporophytic phase terminates with the formation of tetraspores, when a reduction of chromosomes takes place, 20 chromosomes entering each tetraspore. These tetraspores with the reduced number of chromosomes evidently have returned, with respect to nuclear conditions, to the potentialities of the original gametophyte or sexual generation. Again, it may never be possible to grow sporelings from the tetraspore to maturity under experimental conditions, but it is evident that plants derived from the tetraspores must have nuclei with 20 chromosomes. The sexual plants of Polysiphonia are the only forms in which 20 chromosomes are found, therefore it may safely be concluded that the sexual plants arise from tetraspores. The tetraspore then constitutes an indispensable part of the life history of Polysiphonia, and cannot be regarded simply as an accessory type of reproductive structure, such as is illustrated by many forms of asexual spores in the thallophytes or by the gemmae of bryophytes.

To summarize the life history of Polysiphonia, the gametophyte generation begins with the tetraspores and ends with the sexual cells or gametes, whose fusion initiates the sporophyte generation; this covers a long period, including the formation of carpospores, germination of carpospores, development of the tetrasporic plants, and at last ends with the formation of tetraspores. In other words, the sexual plants and the tetrasporic plants present the two distinct phases of an antithetic alternation of generations, with the cystocarp a part of the sporophytic phase, The life history of Polysiphonia may be tabulated as follows:



Taking up the theories concerning the life history of the red algae, OLTMANNS (55) concluded eight years ago, from an investigation of four genera (Dudresnaya, Gloeosiphonia, Callithamnion, and Dasya), that the structure derived from the fertilized carpogonium is comparable to the sporophyte generation of the higher plants. At that time he expressed his opinion definitely that the tetraspore is a special form of reproductive cell, comparable to brood cells or gemmae, and with no fixed place in the life history. However, during the last year (**56**, p. 273) he has admitted the possibilities of reduction phenomena during tetraspore formation.

DAVIS (**24**, pp. 467, 471) in the same year definitely suggested the probability that reduction phenomena would be found in the tetraspore mother cell.

WILLIAMS (**81**) discovered chromosome reduction during tetraspore formation in Dictyota, which led him to conclude that the tetrasporic plant in Dictyotaceae is a sporophyte generation derived from the fertilized egg.

WOLFE (**86**) showed for Nemalion that the cells of the cystocarp have double the number of chromosomes found in the sexual plant, thus presenting the first cytological evidence that the cystocarp of the red algae is sporophytic in character. He places the period of chromosome reduction at the time of carpospore formation, basing his conclusion on a count of chromosomes in the mitosis just previous to the formation of the carpospores. However, he did not report the phenomena characteristic of chromosome reduction, namely the period of synapsis followed by the two mitoses which distribute the chromosomes so as to give a numerical reduction.

Recently STRASBURGER (**75**) has published his views concerning the alternation of generations in the brown algae, remarking that the tetraspores of the red algae seem to be different from those of the Dictyotaceae, and that the place of the chromosome reduction in the red algae should be sought elsewhere than at tetraspore formation, because some of the red algae develop no tetraspores, but instead form monospores. It is true that in some groups of red algae tetraspores are never formed, and in certain of these monospores are present. In these cases chromosome reduction may take place with the formation of the carpospores, or perhaps with their germination, and the monospore when present may have no vital relation to the main cycle of the life history. The group of the red algae is very large and contains a great variety of forms, with a wide range in the complexity of the cystocarp and the vegetative forms, so that it is reasonable to expect important differences in the position of the period of chromosome reduction.

Origin of the tetraspore.—The simplest genera of the red algae, such as Lemanea, Batrachospermum, Chantransia, and Nemalion, have no tetraspores, but some of them have monospores, as in Chantransia and Batrachospermum (including the Chantransia form). In these types the period of chromosome reduction may be associated with the carpospore, either just before its development or at the time of its germination. The monospores, then, in such genera are not vitally concerned with the life history, and indeed are present upon the gametophyte. The tetrasporic plant may have arisen by a suppression of the reduction phenomena in connection with the carpospore, so that it germinates with the sporophytic number of chromosomes, producing a plant with this number, which consequently becomes at once a part of the sporophytic phase. The period of chromosome reduction would be thus postponed from the carpospore to a later period in connection with the newly formed plant. Such plants by developing tetraspores would end the sporophyte generation. It is quite possible that the first tetraspore mother cells corresponded to monospores on the sexual plants except that they had the double number of chromosomes, since such reproductive cells would very naturally become the seat of the delayed reduction phenomena. The resemblance in general morphology of the tetrasporic plants in the red algae to the sexual plants would be expected, because they live under similar environmental conditions, and we have another illustration of such similarity of gametophytes and sporophytes in the Dictyotaceae.

Abnormalities of the nature of monospores.—It should be remembered that sexual plants (cystocarpic) of Polysiphonia occasionally develop an abnormality in the form of a cell resembling a monospore but having the same cell lineage as the tetraspore mother cell. This abnormality may indeed be a reversion to an ancestral type of monospore, that in the process of evolution has given place to the tetraspore mother cell, which is only found in the sporophytic generation. It may be, however, simply an exceptional condition without any phylogenetic significance.

SUMMARY.

The nuclear conditions in the life history of *Polysiphonia violacea* may be summarized as follows:

1. The carpospore on germination shows 40 chromosomes, and 40 chromosomes appear in the vegetative mitoses of the tetrasporic plant; so it may be inferred that the tetrasporic plants come from carpospores.

2. The tetraspore on germination shows 20 chromosomes, and 20 chromosomes appear in the vegetative mitoses of the sexual plant; so it may be inferred that the sexual plants come from tetraspores.

3. The nuclei of the gametes (sperm and carpogonium) contain each 20 chromosomes. The fusion nucleus (sporophytic) in the fertilized carpogonium as a result has 40 chromosomes and gives rise to a series of nuclei in the central cell. Some of these enter the carpospores, which are consequently a part of the sporophytic phase to be continued in the tetrasporic plant. The gametophyte nuclei in the central cell of the cystocarp with 20 chromosomes break down.

4. Tetraspore formation terminates the sporophytic phase with typical reduction phenomena, so that the tetraspores are prepared to develop the gametophyte generation.

5. There is thus an alternation of a sexual plant (gametophyte) with a tetrasporic plant (sporophyte) in the life history of Polysiphonia, the cystocarp being included as an early part of the sporophytic phase.

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EXPLANATION OF PLATES XIX–XXVIII.

The figures were drawn with the aid of an Abbé camera lucida, under Zeiss apochromatic obj. 1.5^{mm} N. A. 1.30, combined with compensating ocular 18; except *figs. 13, 14, 133, 134* drawn with compensating ocular 12; *figs. 103, 109, 114–118, 124, 126, 132, 135, 163–171* drawn with ocular 4; and *figs. 65a, 74a, 147a,–153a, 171a′* drawn with compensating ocular 18 under higher magnification obtained when the tube was extended to the furthest point. The plates are reduced one-half the original size, except *plates XXI, XXII* and *XXVIII*, which are reduced two-fifths.

PLATE XIX.

The first mitosis in the germinating tetraspore.

FIG. 1. Nucleus in resting stage, showing delicate linin network and nucleolus.

FIGS. 2a–2c. Three sections of the same nucleus, showing segregation of chromatin material into about 20 groups, probably prochromosomes, before the formation of chromosomes.

FIGS. 3a, 3b. Two sections of the same nucleus, early prophase; 20 chromosomes may be counted.

FIG. 4. The late prophase; centrosome-like bodies lying at each pole.

FIG. 5. Metaphase showing equatorial plate; nuclear membrane still present; neuclear cavity considerably smaller than in the preceding stage.

FIG. 6. Membrane dissolving; centrosphere-like structures present at the poles of the spindle.

FIG. 7. The polar view of an equatorial plate showing 20 chromosomes and the remains of nucleolar material.

FIG. 8. Anaphase, showing groups of daughter chromosomes assembled at the poles of the spindle.

FIG. 9. Late anaphase, the original nuclear membrane entirely dissolved; centrosphere-like structures passing into vaguely outlined kinoplasmic masses.

FIG. 10. Polar view of late anaphase; space within the group of chromosomes probably indicates the first appearance of nuclear sap within a vacuole.

FIG. 11. Telophase; the membrane of the daughter nucleus is clearly formed.

FIG. 12. Resting condition of daughter nucleus, showing linin network and two nucleoli.

FIG. 13. Cleavage furrow appearing at the periphery of the cell in the middle region.

FIG. 14. The cleavage furrow has penetrated almost to the center of the cell, leaving only a narrow passage admitting of protoplasmic continuity between the daughter cells.

PLATE XX.

The first mitosis in the germinating carpospore.

FIG. 15. Nucleus in the resting condition, showing linin network.

FIGS. 16a–16c. Three sections of the same nucleus, showing 40 prochromosomes.

FIGS. 17a, 17b. Two sections of the same nucleus, early prophase; 40 chromosomes are formed.

FIGS. 18a, 18b. Two sections of the same nucleus; centrosome-like bodies occupying pole.

FIG. 19. Metaphase showing equatorial plate; centrosphere-like structures at the poles of spindle.

FIG. 20. Polar view of an equatorial plate showing group of 40 chromosomes and the remains of nucleolar material.

FIG. 21. Anaphase; groups of daughter chromosomes become assembled at the poles of the spindle.

FIG. 22. Later anaphase; original nuclear membrane entirely dissolved; centrosphere-like structures passing into vaguely outlined protoplasmic masses.

FIGS. 23a, 23b. Two sections of the same daughter nucleus in late anaphase viewed from the pole, showing early stage in development of the vacuoles containing nuclear sap.

FIGS. 24–24c. Three sections of the same daughter nucleus shortly after telophase; nuclear membrane clearly formed and two nucleoli present.

FIG. 25. Resting condition of daughter nucleus, showing linin network and nucleolus.

PLATE XXI.

Mitosis in the vegetative cells of the male plant.

FIG. 26. Apical cell of main filament; resting nucleus of an apical cell, showing linin network.

FIG. 27. The same; network becoming coarser.

Figs. 28–35. Apical cells of developing hairs.

FIG. 28. Chromatin granules aggregated into 20 prochromosomes.

FIG. 29. Prophase; 20 chromosomes clearly present; a centrosome-like body at each pole.

FIG. 30. Metaphase showing equatorial plate; centrosphere-like structures at the poles of spindle.

FIG. 31. The polar view of equatorial plate showing 20 chromosomes.

FIG. 32. Late metaphase; two groups of daughter chromosomes just separating.

FIG. 33. Anaphase; two groups of daughter chromosomes separated by a large vacuole which enters between them.

FIG. 34. Telophase; nuclear membrane around the daughter nuclei.

FIG. 35. Later stage than the previous figure; cleavage furrow proceeding inward so as to divide the apical cell.

Mitosis in the vegetative cells of female plants.

Figs. 36–42. Apical cells of developing hairs.

FIG. 36. Prophase of nucleus; centrosome-like body present at the pole.

FIG. 37. Metaphase, showing equatorial plate; centrosphere-like structure conspicuous.

FIG. 38. The polar view of metaphase.

FIG. 39. Late metaphase.

FIG. 40. Anaphase.

FIG. 41. Anaphase; the vacuole beginning to intrude between the groups of daughter chromosomes.

FIG. 42. Telophase; cleavage furrow appearing between the two daughter nuclei.

FIG. 43. Mitotic figure in the old region of thallus, showing large centrosphere-like structures.

FIG. 44. Polar view of the same stage as the above, showing 20 chromosomes.

Mitosis in the vegetative cells of the tetrasporic plant.

Figs. 45–51. All apical cells of main filament.

FIG. 45. Resting nucleus, showing linin network.

FIG. 46. Chromatin granules commence to aggregate to form prochromosomes.

FIG. 47. Early prophase showing 40 chromosomes.

FIG. 48. Metaphase showing equatorial plate; centrosphere-like structures present.

FIG. 49. Late metaphase; groups of daughter chromosomes separated.

FIG. 50. Polar view of metaphase showing 40 chromosomes.

FIG. 51. Anaphase; vacuole intruding between two groups of daughter chromosomes.

PLATE XXII.

Spermatogenesis.

Figs. 52–61. Formation of the sperm mother cell.

FIG 52. Terminal portion of a very young antheridium, consisting of an axial siphon of three cells; the nucleus of the lower cell is in a resting condition; the middle one in early prophase; and the upper one in anaphase.

FIG. 53. Mitotic figure in a cell of the axial siphon to form a stalk cell laterally.

FIG. 54. A cell of the axial siphon with the stalk cell formed laterally.

FIG. 55. Prophase in the stalk cell showing 20 chromosomes.

FIG. 56. Metaphase showing equatorial plate; centrosphere-like structures present.

FIG. 57. Polar view of the same stage as in the preceding figure, showing 20 chromosomes.

FIG. 58. Late metaphase stage; nuclear membrane still present.

FIG. 59. Anaphase; vacuole intruding between two groups of daughter chromosomes.

Fig. 60. Still later stage of anaphase.

FIG. 61. Telophase; cleavage furrow separating the sperm mother cell from the stalk cell.

Figs. 62–74. Formation of the first sperm.

FIG. 62. Resting nucleus of sperm mother cell, showing linin network.

FIG. 63. Fine reticulum transformed into 20 prochromosomes.

FIG. 63a. Portion of *fig. 63* under higher magnification.

FIG. 64. Prophase; a few spindle fibers attached to the chromosomes; centrosome-like bodies at the poles.

FIG. 64a. Portion of *fig. 64* under higher magnification.

FIG. 65. Metaphase; the centrosphere-like structures evident.

FIG. 65a. The nucleus of *fig. 65* under higher magnification.

FIG. 66. The polar view of the stage shown in the previous figure; 20 chromosomes clearly present.

FIG. 67. Late metaphase.

FIG. 68. Anaphase; vacuole intruding between the two groups of daughter chromosomes.

FIG. 69. Later stage of anaphase.

FIG. 70. Lower nucleus passing into resting condition.

FIG. 71. Cleavage furrow separates the sperm above from the sperm mother cell which is destined to produce later a second sperm.

FIG. 72. Sperm almost formed; the sperm mother cell beginning to elongate, preliminary to the development of a second sperm

FIG. 73. Side view of mature sperm

FIG. 74. Sperm viewed from above; chromosomes maintaining their individual forms.

FIG. 74a. Portion of *fig. 74* under higher magnification.

Figs. 75–81. Formation of the second sperm.

FIG. 75. Prophase of mitosis to form second sperm; the first sperm still present at the side, so that the sperm mother cell assumes an asymmetrical outline.

FIG. 76. Metaphase showing equatorial plate; centrosphere-like structures present.

FIG 77. Polar view of same stage as in previous figure.

FIG. 78. Late metaphase.

FIG. 79. Anaphase.

FIG. 80. Later anaphase; vacuole between the two groups of daughter chromosomes.

FIG. 81. Cleavage furrow separating the second sperm from the sperm mother-cell.

PLATE XXIII.

The formation of the procarp.

FIG. 82. Mitotic figure in the cell of the central siphon of a procarp, to form the first peripheral cell; centrosphere-like structures present.

FIG. 83. Polar view of the same mitosis, showing 20 chromosomes.

FIG. 84. First peripheral cell is formed.

FIG. 85. Mitosis to form second peripheral cell.

FIG. 86. Polar view of the same mitosis; 20 chromosomes present.

FIG. 87. Anaphase of the same mitosis.

FIG. 88. The fifth peripheral cell, called the pericentral cell, formed on the left of the central siphon; this cell is destined to develop the carpogonial branch.

FIG. 89. Mitosis to form first cell of the carpogonial branch from the pericentral cell.

FIG. 90. Polar view of the same stage as the previous figures, showing 20 chromosomes.

FIG. 91. Anaphase.

FIG. 92. Late anaphase.

FIG. 93. Telophase; vacuole intruding between the daughter nuclei.

FIG. 94. Mitotic figure to form second cell of the carpogonial branch; *pc*, pericentral cell.

FIG. 95. Polar view of the same stage as shown in the previous figure; 20 chromosomes readily seen.

FIG. 96. Mitotic figure forming the third cell of the carpogonial branch.

FIG. 97. Polar view of the same stage.

FIG. 98. Mitotic figure to form the fourth cell of the carpogonial branch.

FIG. 99. Polar view of the previous figure.

FIG. 100. Mitosis of the nucleus within the fourth cell of carpogonial branch, which develop the trichogyne and carpogonium; this mitosis gives two daughter nuclei as shown in *fig. 101*; being cut obliquely, the chromosomes, 20 in number, may be counted.

FIG. 101. The two nuclei within the carpogonium or the fourth cell of the carpogonial branch: *pc*, pericentral cell.

PLATE XXIV.

Development of the trichogyne.

FIG. 102. Development of the trichogyne from the carpogonium.

FIG. 103. A mature procarp in section, showing the situation of the carpogonial branch: *pc*, pericentral cell; *as*, axial siphon.

FIG. 104. Carpogonium with trichogyne at maturity; note the difference in size between the sister nuclei, for the carpogonium with its nucleus has enlarged greatly.

Fertilization.

FIG. 105. Sperm attached at the tip of the trichogyne.

FIG. 106. Contacts of sperm, its nucleus passing into trichogyne; note the individual chromosomes in the sperm nucleus.

FIG. 107. Sperm cell emptied of contents; sperm nucleus about to pass the trichogyne nucleus.

FIG. 108. Sperm nucleus having passed the trichogyne nucleus is about to enter the carpogonium.

FIG. 109. Section of procarp showing the situation of the carpogonium at the time of fertilization.

FIG. 109a. The carpogonium of *fig. 109* under higher magnification; sperm nucleus about to fuse with the female nucleus, which has moved upwards in the carpogonium.

FIG. 110. Mitotic figure in the formation of an auxiliary cell from the pericentral cell, which takes place parallel with the nuclear fusion in the carpogonium.

FIG. 111. Prophase of the above mitosis showing 20 chromosomes.

FIG. 112. Mitosis in a cortical cell of the procarp.

FIG. 113. Polar view of the above mitosis, showing 20 chromosomes.

FIG. 114. Section of procarp showing situation of the carpogonium at the time of contact between the sperm and carpogonium nuclei.

FIG. 114a. The carpogonium of *fig. 114* under higher magnification; the sperm nucleus beginning to fuse with the female; no membrane observable around the sperm nucleus since the beginning of entry of it into the trichogyne; the female nucleus in resting condition.

FIG. 115. Section of procarp showing carpogonium at a late stage in the fusion of the sexual nuclei.

FIG. 115a. The carpogonium of *fig. 115* under higher magnification; the chromosomes of the sperm nucleus beginning to separate in the female nucleus; the network in female nucleus preparing to form chromosomes.

FIG. 116. The fusion nucleus in the carpogonium after the female chromosomes have been formed, showing a mingling of male and female; the total number of chromosomes is 40.

Development of the cystocarp.

FIG. 117. Section of procarp at the time of the first mitosis of the fusion nucleus within the carpogonium.

FIG. 117a. The carpogonium of *fig. 117* under higher magnification, showing the first mitosis of the fusion nucleus.

FIG. 118. Procarp showing the first mitosis of the fusion nucleus in the carpogonium; cut obliquely.

FIG. 118a. The carpogonium of *fig. 118* under higher magnification, showing a polar view of first mitosis of the fusion nucleus; 40 chromosomes present.

FIG. 119. Prophase of a nucleus within the first cell of the carpogonial branch.

FIG. 120. Metaphase of the above mitosis.

FIG. 121. Polar view of the same mitosis showing 20 chromosomes.

FIG. 122. Anaphase of the same mitosis.

PLATE XXV.

FIGS. 123a, 123b. Two sections of the fusion nucleus at metaphase of the first mitosis.

FIG. 124. Section of the procarp at anaphase of the first mitosis of the fusion nucleus within the carpogonium.

FIG. 124a. The carpogonium of *fig. 124* under higher magnification.

FIG. 125. Migration of one of two daughter nuclei (sporophytic), resulting from the first mitosis of the fusion nucleus, into the third auxiliary cell (compare with *a3* in *diagram 3*).

FIG. 126. Migration of two daughter nuclei (sporophytic) into the pericentral cell, the third auxiliary cell having fused with it; a nucleus from one of the auxiliary cells below also entering the pericentral cell; the first, second, and third cells of the carpogonial branch, together with the carpogonium, about to collapse.

FIG. 127. Mitoses of two sporophytic nuclei derived from the fusion nucleus and of a gametophytic nucleus also within the central cell; the distinction between

the mitotic figures is readily seen by estimating the number of chromosomes arranged in the equatorial plate.

FIG. 128. Sporophytic nucleus in a process from the central cell, from which after a mitosis a carpospore will be formed; the nucleus in prophase; 40 chromosomes may be counted.

FIG. 129. Metaphase of the mitosis which separates the carpospore from the stalk cell.

FIG. 130. Polar view of the same mitosis cut obliquely, showing 40 chromosomes.

FIG. 131. Telophase of the same mitosis; the upper nucleus will lie in the carpospore.

FIG. 132. Section of a cystocarp, showing central cell (*cc*), stalk cell (*sc*), carpospore (*sp*), and paranematal filaments (*pf*).

FIG. 133. Stalk cells beginning to fuse together side by side, and their nuclei, with other nuclei remaining in the central cell, beginning to disorganize.

FIG. 134. Disorganizing nuclei in the central cell.

FIGS. 135a–135d. Four sections of a procarp, showing the arrangement of the auxiliary cells (compare with *diagram 3*); the fusion nucleus in the carpogonium (*carp*) shows the mingling of male and female chromosomes.

PLATE XXVI.

The tetraspore formation.

FIG. 136. Prophase in a cell of the central siphon previous to the formation of pericentral cell, showing 40 chromosomes.

FIG. 137. Metaphase of this mitosis.

FIG. 138. Anaphase.

FIG. 139. Polar view of anaphase; 40 chromosomes present.

FIG. 140. Tip of a filament, showing stages in the formation of the tetraspore mother-cell; *pc*, pericentral cell; *sc*, stalk cell; *tmc*, tetraspore mother cell.

FIG. 141. Chromatin network in resting nucleus of pericentral cell.

FIG. 142. Metaphase of the mitosis in the pericentral cell; centrosphere-like structures present.

FIG. 143. Late metaphase of the same.

FIG. 144. Anaphase.

FIG. 145. Telophase; vacuole intruding between the daughter nuclei.

FIG. 146. Tetraspore mother cell becoming separated from the stalk cell by the cleavage furrow.

FIG. 147. Later stage of the above; tetraspore mother cell increased in size; the nucleus with delicate linin network.

FIG. 147a. Portion of linin reticulum in *fig. 147* under higher magnification.

FIG. 148. Reticulum becoming transformed into a coarser chromatin network with here and there large knots upon the threads.

FIG. 148a. Portion of network from *fig. 148* under higher magnification.

FIG. 149. Irregular network becoming transformed into more even threads.

FIG. 149a. Portion of threads from *fig. 149* under higher magnification.

FIG. 150. Threads becoming arranged parallel to one another.

FIG. 150a. Portion of threads from *fig. 150* under higher magnification.

FIG. 151. Synapsis.

FIG. 151a. Portion of the parallel threads from *fig. 151* fusing in parts, under higher magnification.

FIG. 152. Nucleus emerging from synapsis.

FIG. 152a. Portion of fused parallel threads or spirem from *fig. 152* under higher magnification; the spirem beginning to split longitudinally.

FIG. 153. Later stage than the above; spirem beginning to segment to form chromosomes; the split segments arranged side by side.

FIG. 153a. Segments of spirem from *fig. 153* under higher magnification, showing X and double L and V forms.

PLATE XXVII.

FIG. 154. Early prophase of mitosis in tetraspore mother cell; 20 bivalent chromosomes are present.

FIG. 154a. Chromosomes from *fig. 154* under higher magnification, showing bivalent character.

FIG. 155. Later prophase; bivalent chromosomes have been shortened.

FIG. 156. Metaphase; a great number of chromosomes (about 80) arranged in the equatorial plate; this large number results from the premature division of the halves of the bivalent chromosomes shown in *fig. 155;* the 80 chromosomes are therefore granddaughter chromosomes, which will be distributed to the four tetraspores; centrosphere-like structures poorly differentiated.

FIG. 157. Later metaphase of the first mitosis; two groups of daughter chromosomes, 40 in each, just separating and the four poles of second spindle already differentiated.

FIG. 158. Metaphase of second mitosis; four groups of granddaughter chromosomes (three seen) passing to the poles.

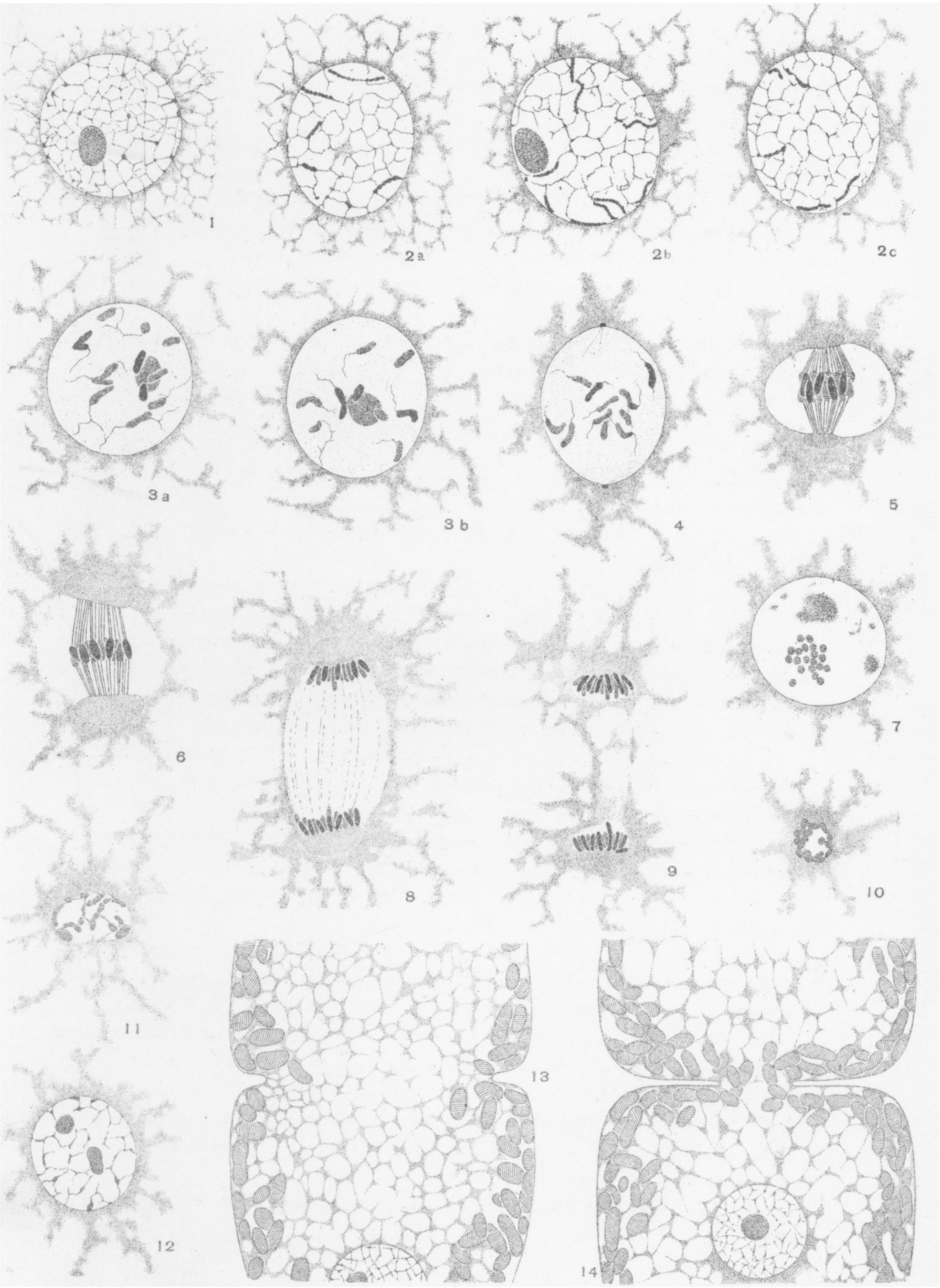
FIG. 159. Groups of granddaughter chromosomes at the poles; nucleolus has appeared in the center of the nuclear space.

FIG. 160. Groups of granddaughter chromosomes forming chromatin network; membrane surrounding the nuclear cavity assuming a tetrahedral shape with kinoplasm accumulating at the corners.

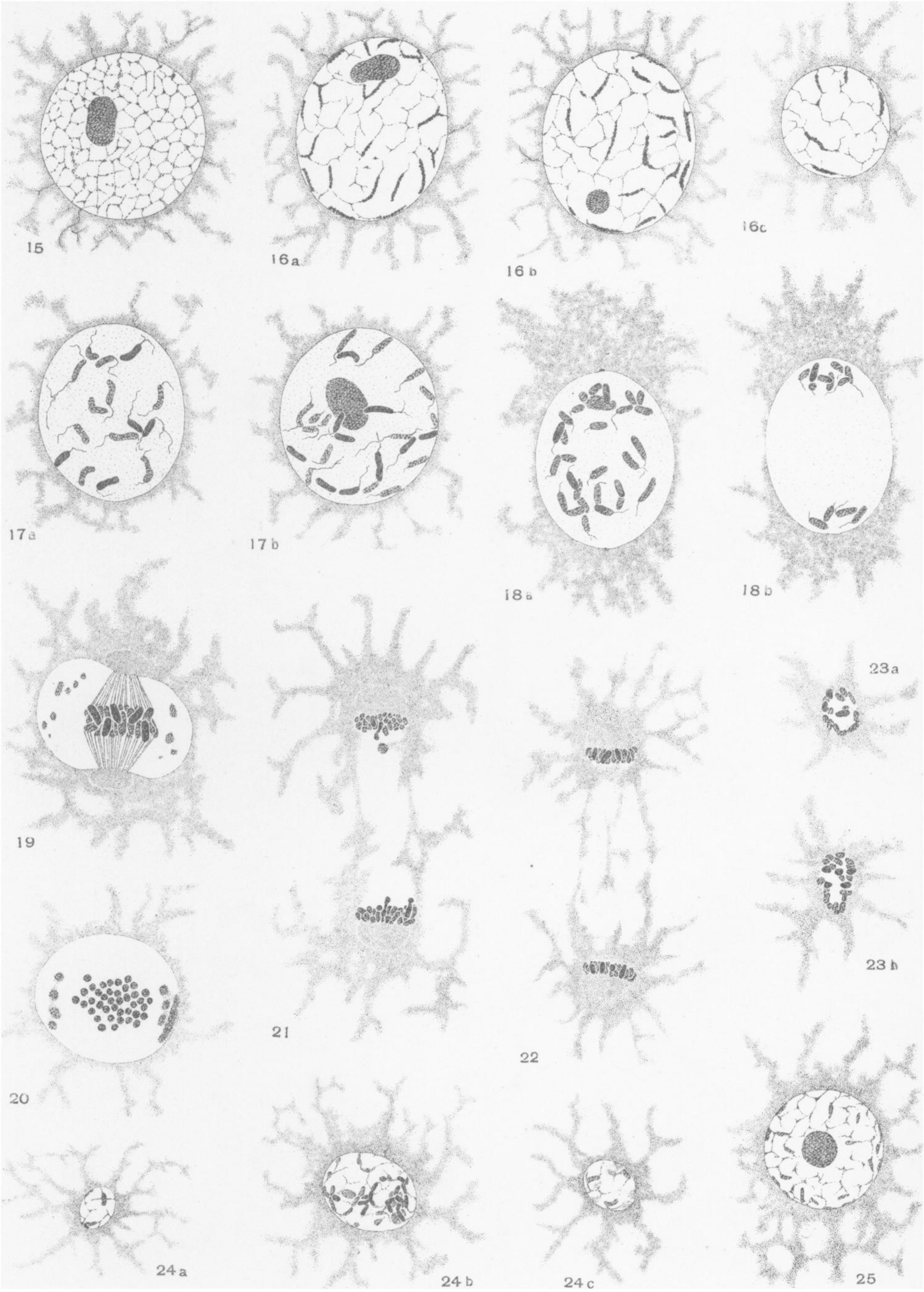
FIG. 161. Groups of granddaughter chromosomes have formed an anastomosing chromatin network; membrane surrounding nuclear cavity has broken in the region between the four poles so as to admit the entrance of kinoplasmic fibrils without the membrane; the fibrils grow toward the center of the nuclear cavity.

FIG. 162. Daughter nuclei becoming separated by the growth of kinoplasm which surrounds the chromatin network.

FIG. 163. Tetraspore mother cell at the time of separation of the daughter nuclei.



YAMANOUCHI on POLYSIPHONIA



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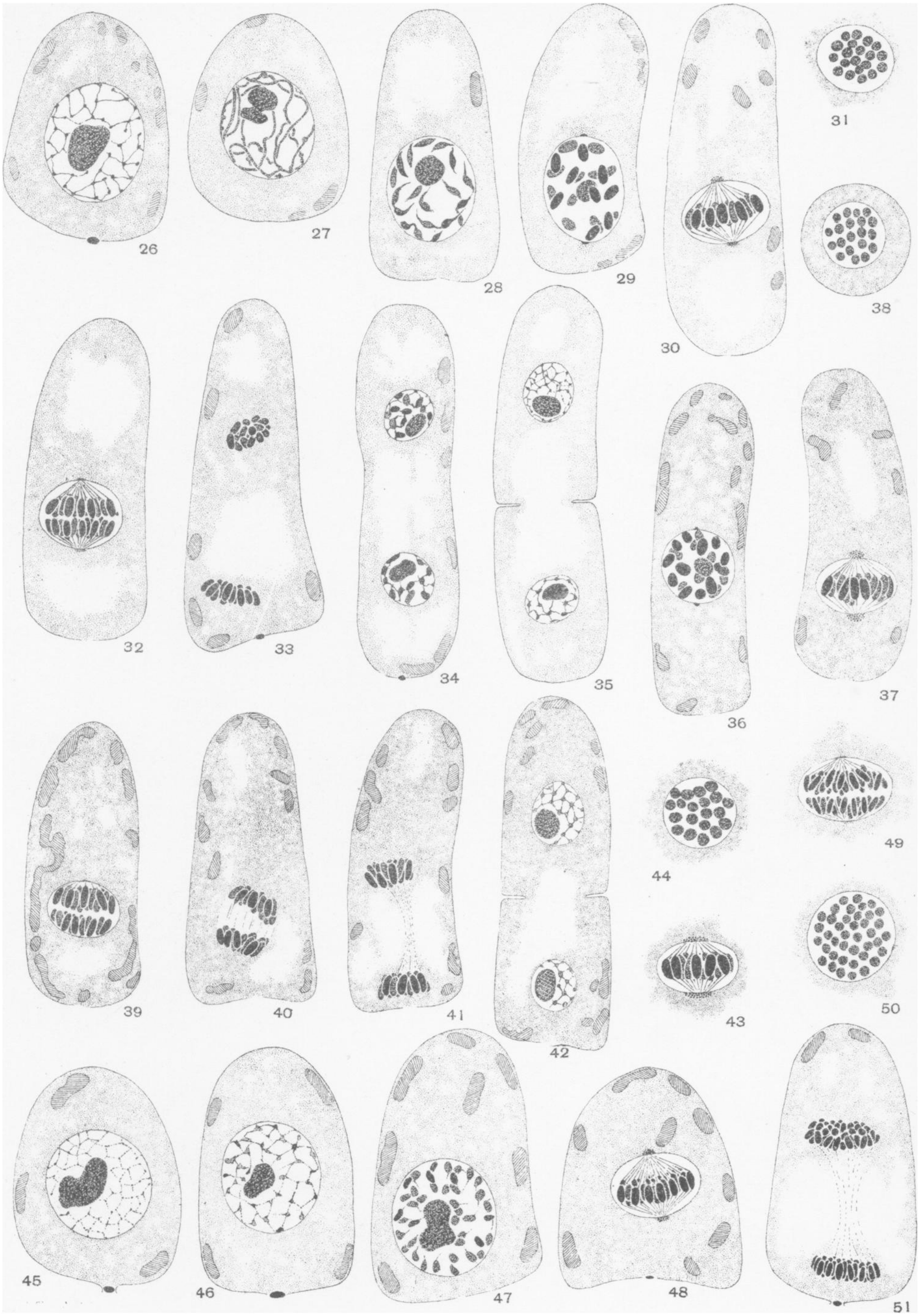

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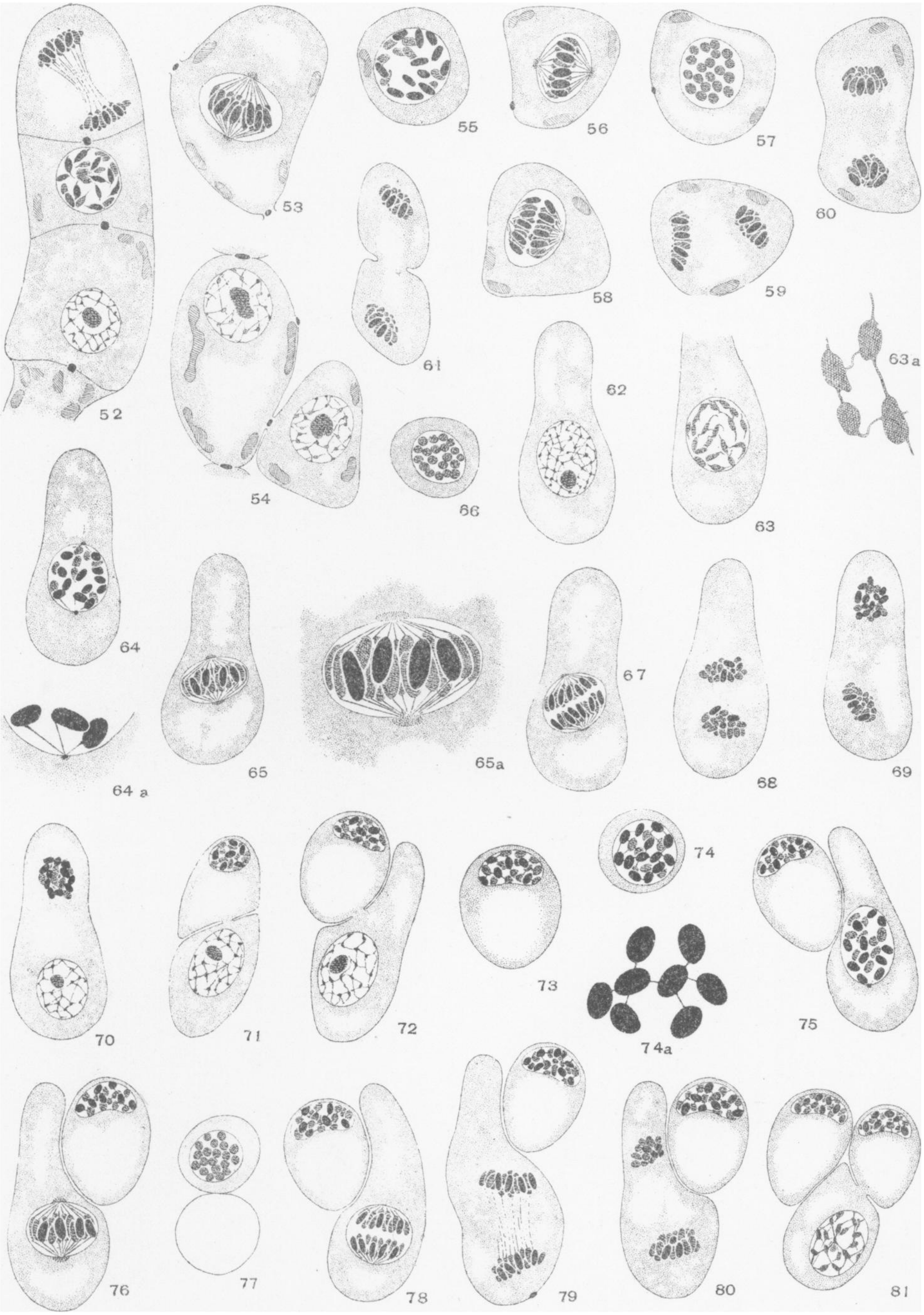

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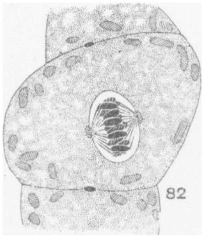
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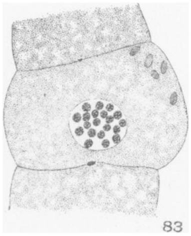
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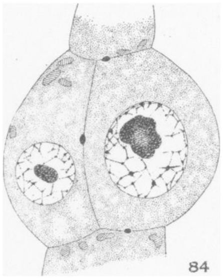
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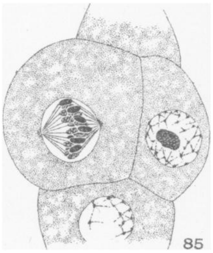
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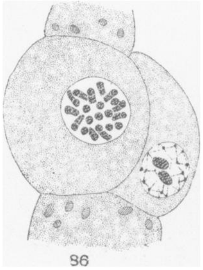
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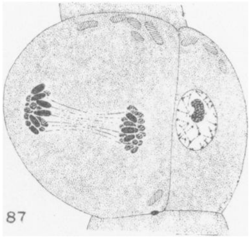
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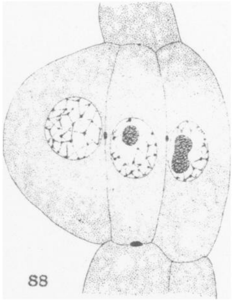
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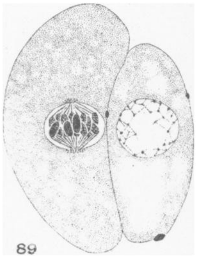
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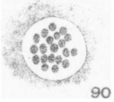
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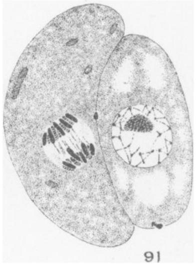
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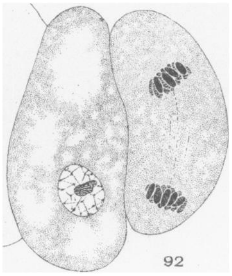
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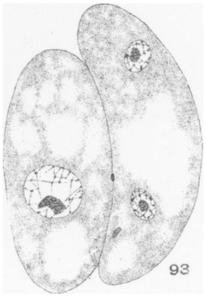
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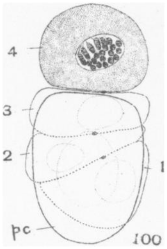

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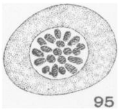
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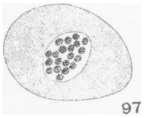
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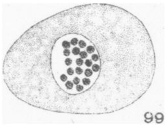
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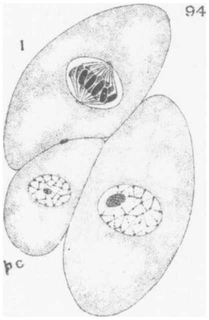

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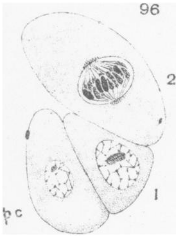

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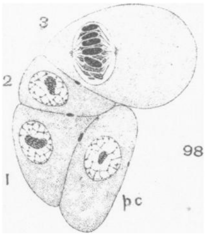

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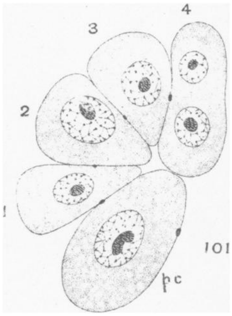

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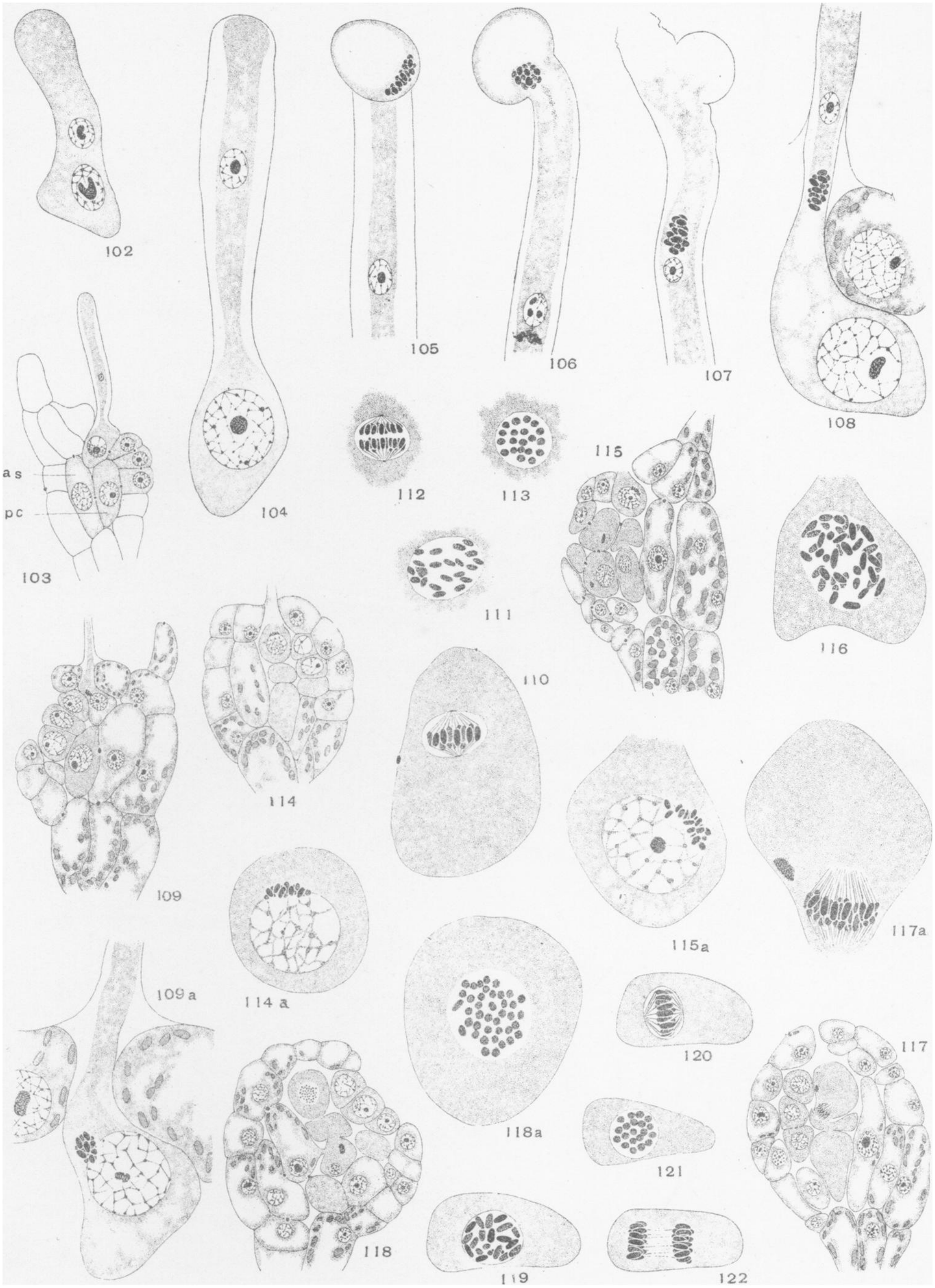
PLATE XXIV



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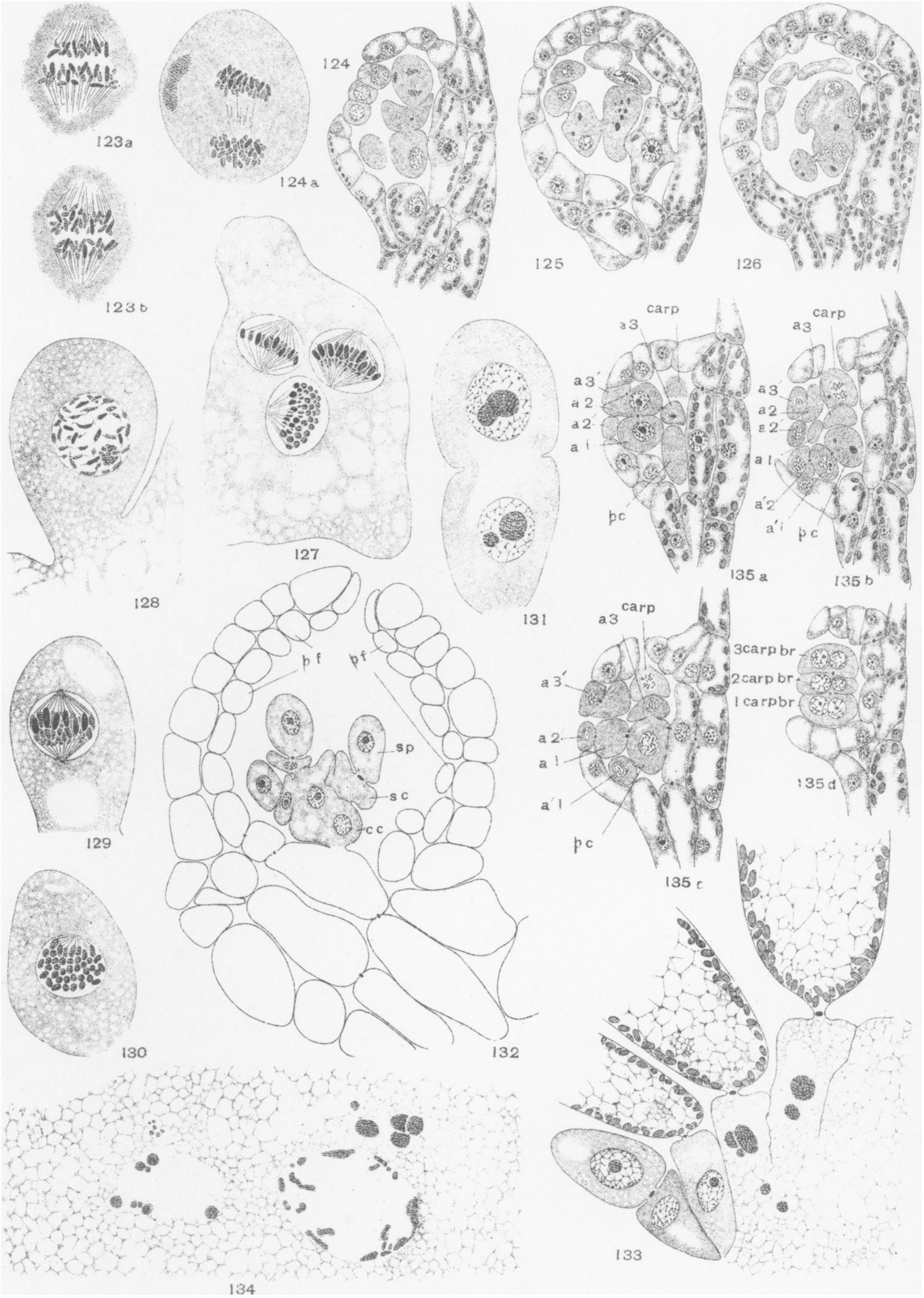

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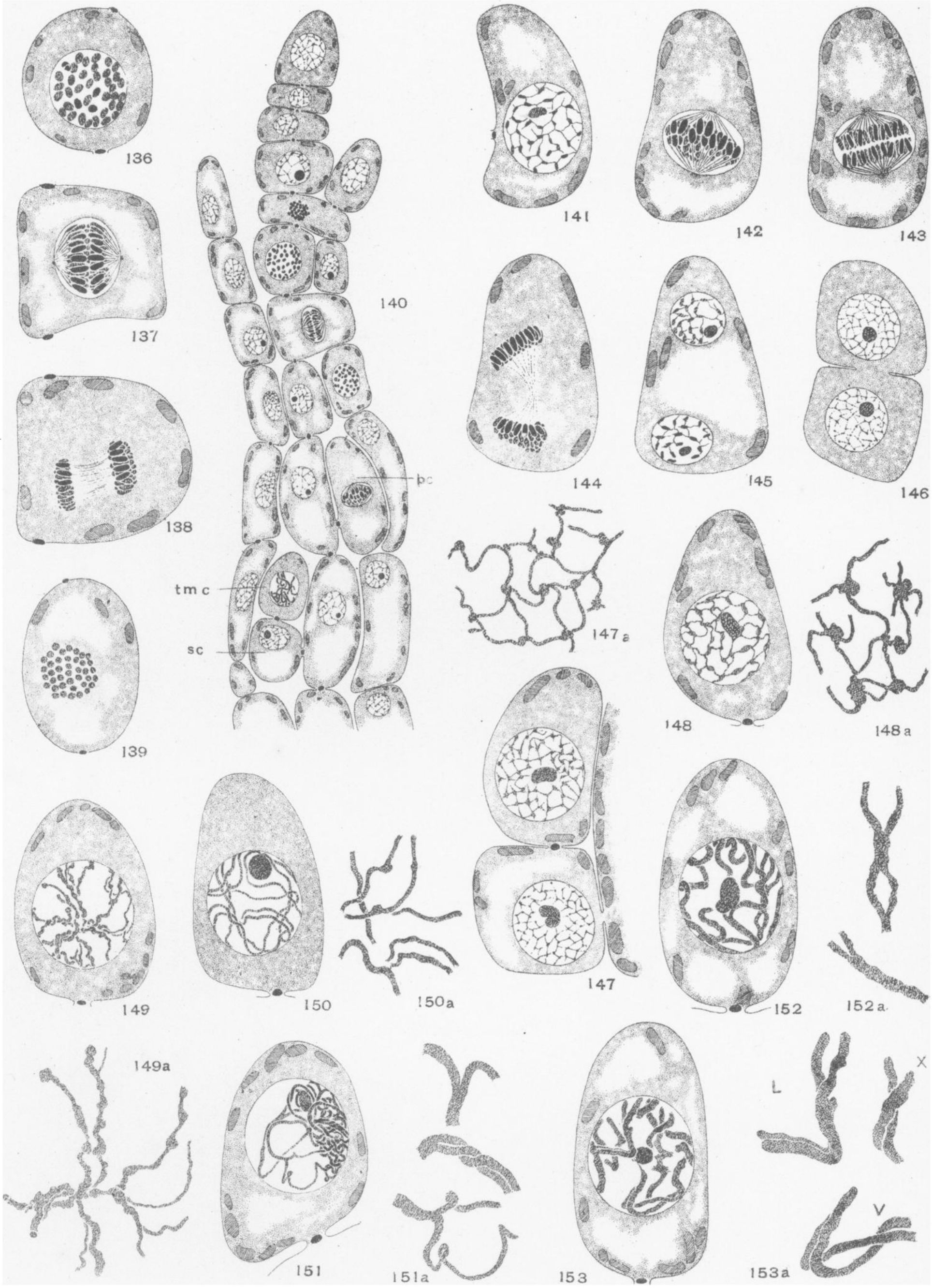

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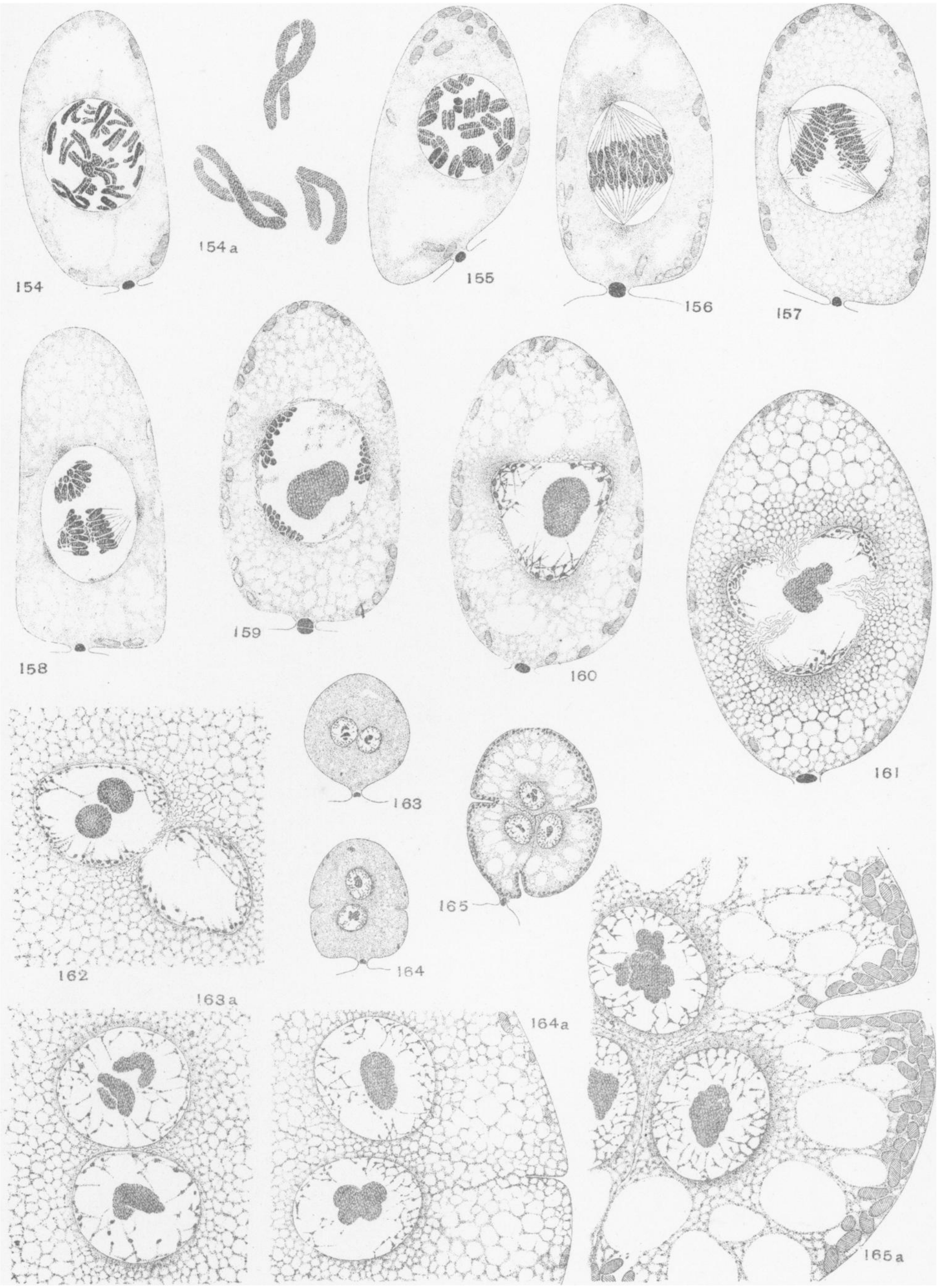

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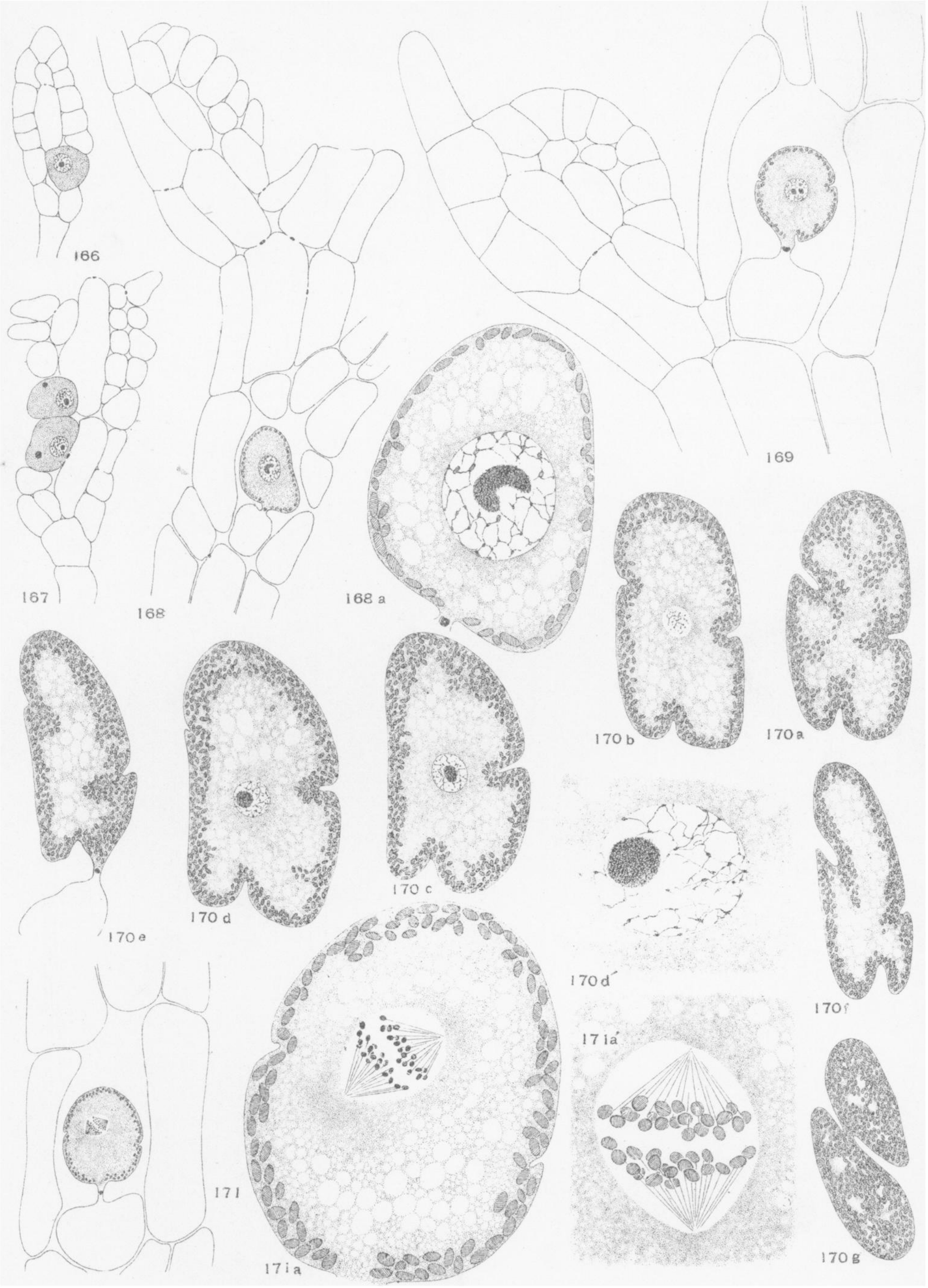

YAMANOUCHI on POLYSIPHONIA

FIG. 163a. The nuclei of *fig. 163* under higher magnification, showing their structure with linin network and nucleoli.

FIG. 164. The four daughter nuclei are completely formed (two visible); cleavage furrow appearing at the periphery.

FIG. 164a. Portion of *fig. 164* under higher magnification, showing details of cleavage furrows.

FIG. 165. Tetraspore mother cell with cleavage furrows growing towards the center.

FIG. 165a. Portion of *fig. 165* under higher magnification, showing the vacuolar structure and the progress of the cleavage furrows.

PLATE XXVIII.

Abnormalities.

FIG. 166. A young antheridium in which a stalk cell has increased in size and assumed the appearance of an auxiliary cell in the procarp.

FIG. 167. Showing the division of a stalk cell; the black spot within the cell indicates transverse section of protoplasmic strands connecting it with other stalk cells above or below.

FIG. 168. A cell formed on a sexual plant whose lineage is similar to that of the tetraspore mother cell.

FIG. 168a. This cell from *fig. 168* under higher magnification; the nucleus in a resting condition.

FIG. 169. Still later stage in the formation of this cell.

FIGS. 170a–170g. Seven sections of a similar cell; nucleus remains undivided, although the beginning of a cleavage furrow is present, which however never proceeds further.

FIG. 170d′. Nucleus of *fig. 170d* under higher magnification, in a resting condition.

FIG. 171. A rare case where the nucleus of this cell undergoes a typical mitosis.

FIG. 171a. The nucleus of *fig. 171* under higher magnification.

FIG. 171a′. The mitotic figure of *fig. 171a* under still higher magnification shows that this division is typical and that the daughter chromosomes are 20 in number in each group.